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On the Water Relations of Plant Cells

BY

D. THODAY

(*From the Department of Botany, University College of North Wales, Bangor*)

With one Figure in the Text

IN a recent paper Burström (1948) has maintained that, when a vacuolated cell is absorbing water, the turgor pressure must be greater than the wall pressure since the wall is being further distended. Surely there is a confusion of thought here. Since action and reaction are equal and opposite, the pressure of the wall and the hydrostatic pressure of the contents must be equal at all stages in the process. Whatever brings about the increase in wall pressure causes also the increase in hydrostatic pressure. The case is analogous to that of a pneumatic tyre being pumped up: the air pressure and the opposing pressure of the distended tyre are equal and opposite at every stage and both are increased together by the action of the pump.

Similarly, in Pfeffer's osmotic apparatus, before the pressure in the manometer reaches the maximum value which, by definition, measures the osmotic pressure of the solution, the manometer measures at any intermediate stage the hydrostatic pressure already reached in the osmotic cell at that stage: this pressure is equal and opposite to the manometric pressure and to the back pressure of the wall of the vessel and its membrane on the solution. The lack of equilibrium which renders the system a dynamic one is due to the osmotic *potentialities* of the solution.

To resolve the confusion all that seems necessary is to recognize that osmotic pressures and suction pressures (likewise imbibition pressures) are potentials, which are only made evident by actual pressures under appropriate conditions (cf. Beck, 1928, p. 415). In a cell at the stage of incipient plasmolysis, or in a cell in air that has lost water by evaporation till the wall is just completely relaxed, neither wall pressure nor turgor pressure exists, but the osmotic potential of the cell is available as a suction potential if the cell is put into effective communication with a source of water at a lower suction potential than its own. The difference of suction potential plays a part analogous to that of the tyre-pump. The maximum pressure that the tyre-pump will provide corresponds to the osmotic pressure of the solution, which expresses the limit of effectiveness of its osmotic potential.

When a cell has reached equilibrium with a solution in which it is immersed, the osmotic potential of the cell-sap is in part balanced by the osmotic potential of the external solution, in part expressed in the turgor pressure which is balanced by the wall pressure. If the cell is removed from the

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solution, the former part is available as a suction potential which becomes effective when the cell is immersed in water.

These relations hold whatever the nature of the potential differences that come into play. If, as appears to be indicated by Bennet-Clark's experiments, cells can hold water against an external solution of higher osmotic pressure than that of their sap, there must exist in addition to ordinary osmotic potentials other potentials, such as electrosmotic potentials maintained by the activity of the protoplasm. The suction potential of the cell would then be of composite origin; but the equilibrium relations could still be expressed by the usual equations, for example,

$$P-T=p=S,$$

where P represents the potential of the cell (in this case partly osmotic, partly electrosmotic), p that of the external solution, T the turgor pressure, and S the suction potential of the cell. Alternatively a separate expression could be added, say, E, for the additional factor, leaving P for the ordinary osmotic potential, so that $(P+E)-T=p=S$.

When, however, we attempt to envisage the physical meaning of the balanced potentials at the molecular level, we are on less sure ground. The osmotic potential is related to the concentration of the solute molecules and ions but independent of their natures. Electrosmotic potentials depend on the nature of the solvent and of the membrane permeable to it, which determine the electrical charge on the solvent molecules in the membrane; also on the processes that maintain the difference of electrical potential between the two sides of the membrane. The situation is evidently complex. It is not, however, necessary to adopt particular views of these mechanisms in order to express the hydrostatic conditions that result from their action.

Meyer (1938) prefers the concept of water 'diffusion pressure' to osmotic pressure or suction pressure as having particular reference to the solvent. Taking the diffusion pressure of pure water as the standard of reference, he substitutes 'diffusion pressure deficit' for osmotic pressure. This term is also liable to be misleading unless it is recognized that this also signifies a potential which, like osmotic potentials and suction potentials, is measured in terms of hydrostatic pressures, after establishing equilibrium conditions in which the potential difference is nil. Meyer, in fact, defines 'diffusion pressure deficit' as equalling the external pressure required to raise the diffusion pressure of the solution to that of the pure solvent.

'Diffusion pressure' has the merit that it is related directly to the pressure of water vapour which would be in equilibrium with the solution or cell. This relation is made use of in Barger's well-known method for determining osmotic pressures of small quantities of fluid, on the principle that solutions in equilibrium with the same pressure of water vapour would be in osmotic equilibrium with one another if brought into communication through a semipermeable membrane. Vapour pressure being taken as an index, the water 'diffusion pressure' of a solution will be increased by hydrostatic pressure:

thus in a fully turgid cell the turgor pressure increases the water diffusion pressure from that of the uncompressed sap to that of pure water. How this change is to be envisaged at the molecular level is again another question.

Burström states (p. 61) that 'In the cell we have only two actual pressures, the turgor pressure, equalling the difference in D.P. (diffusion pressure) of the water on the two sides of the membrane, and the wall pressure, caused by the tension in the membrane. These two pressures are of fundamentally different physical origin, and each may attain any value independent of the other.' Here he confuses the issue by departing from the customary use of the term turgor pressure and applying it to the difference of diffusion potential which is not a pressure in the same sense. It is able to develop a pressure and do work under appropriate conditions; but the only two actual pressures in the strict, hydrostatic sense are the wall pressure and the turgor pressure, which are equal and opposite. Burström also states (p. 60) that when a sugar solution is separated from water by a movable semipermeable membrane, the pressure difference causing the membrane to move can be computed and equals the diffusion pressure deficit. There is a fallacy here. The pressure moving the membrane is *caused* by the 'diffusion pressure deficit' or difference of osmotic potential, but the two are not identical. The effective pressure will depend on the frictional resistance offered to the movement of the membrane. To measure the 'diffusion pressure deficit' it would be necessary to measure the resistance offered by the membrane to the movement of water and also the rate of flow. The case is analogous to that of an electrical system in which the difference of electric potential can be determined if the current and the resistance can be measured, from the relation $C = \frac{E}{R}$.

Similarly, when the potential difference is known and the rate of flow can be measured, the resistance to flow can be obtained. This is the principle of the plasmolytic method for determining the permeability of protoplasm, which is inversely proportional to the resistance.

The point of view here stated may be conveniently summarized by reference to the three methods available in theory for determining the condition of a plant cell in relation to the retention or absorption of water:

1. The pressure of water vapour in equilibrium with it may be determined. The suction potential of the cell is equal to the osmotic potential of solutions with the same vapour tension.

2. The cell may be compressed and the pressure noted at which water just begins to exude: if in this condition the cell were brought into contact with pure water it would neither take up water nor yield water.

The equilibrium would be represented by the equation:

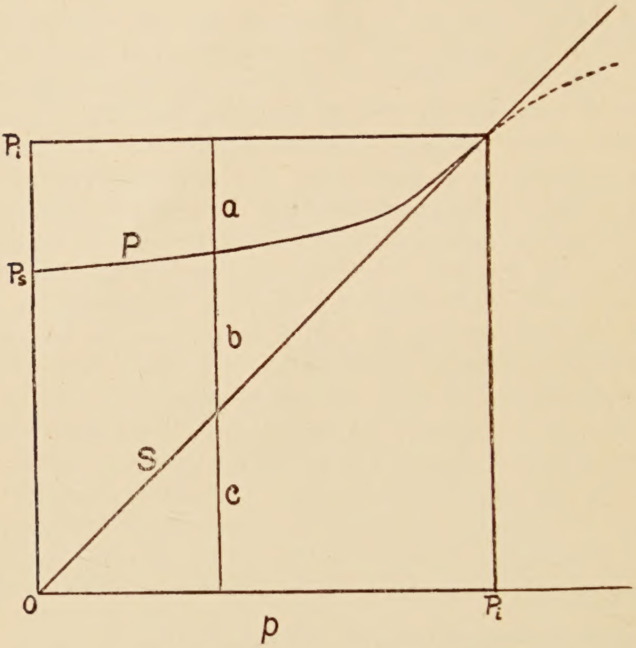
$$P - W - e = 0,$$

where P = the osmotic potential of the cell-sap, W = the initial wall pressure, e = the pressure applied externally.

The hydrostatic pressure within the cell would be $W+e$.[1]

The water diffusion pressure of the system would be equal to that of pure water, the diffusion pressure deficit nil.

Further compression would lead to exudation of water and an increase in concentration of the sap, P increasing therefore to balance the increase in e.



The abscissae represent the osmotic potential of the external solution, p, rising from zero to P_i, the osmotic potential of the cell-sap at incipient plasmolysis. Ordinates represent cell potentials. S, the suction potential, is equal to p. P varies from P_i at incipient plasmolysis (on the right) to a value at full turgor depending on the degree of dilution, i.e. on the expansion of the volume of the vacuole: the saturation value $P_s = P_i \times \frac{V_i}{V_s}$.

The form of the P-curve between these two points will depend on the exact course of volume-change. Tamiya points out that whereas Ursprung and Blum assumed proportionality between increase of volume and turgor pressure, measurements by various experimenters show greater extensibility at low turgor pressures and increasing resistance to further distension as turgor increases. The degree of curvature is therefore greater to the right, and diminishes to the left as full turgor is approached.[2]

At any intermediate point between $p = 0$ and $p = P_i$, the ordinate is divided by the P- and S-curves into three portions. Of these, $c = S$, $b = P-S$ = the turgor pressure, a is the loss of osmotic potential of the sap resulting from dilution.

---

[1] Actually this is a simplification. Compression would increase the hydrostatic pressure, which would involve an increase in the wall pressure in directions other than the direction of compression. Tissue tensions involve similar relations. In a tissue confined within a resistant layer, the latter exerts a pressure on the confined tissue, but the cell-walls where they abut on air spaces bulge into the spaces and there exert a back pressure on the cell contents equal and opposite to the full hydrostatic pressure within the cell.

[2] The P-curve in the figure has been recalculated from data obtained for *Nitella* by Stow quoted by Tamiya (1938).

3. A solution of a suitable solute (usually sucrose) may be found of such a concentration that no water passes either way when the cell is immersed in it. In this case the equilibrium would be represented by the equation:

$$P - W = p,$$

where p = the osmotic potential of the external solution.

Here the hydrostatic pressure in the cell is the original turgor pressure $T = W$.

The diffusion pressure deficit is equal to p.

In both 2 and 3, at equilibrium, the 'net suction pressure' of the system (Stiles, 1924, p. 98), i.e. the potential difference, is nil; but whereas the suction potential of the cell in 2 is zero, in 3 it is equal to p.

If a difference of potential exists between the cell and its environment it will cause movement of water into or out of the cell. Though the conditions are changing, the condition of the cell at any given moment can nevertheless be expressed in terms of a similar hypothetical equilibrium. The suction potential of the cell, S, will be $P - W$; the difference of potential, or net suction pressure, will be $S - p$.

The extent of the changes occurring in the attainment of equilibrium depends on the extensibility of the cell wall. This aspect has been specially considered by Sakamura (1937) and Tamiya (1938) and expressed in a novel form of diagram, which is reproduced here from Tamiya with modified lettering.

As pointed out by these and other authors, the validity of the relations expressed depends on the temperature and the value of P_i remaining constant.

For cells actually immersed in solutions the diagram can be extended to the right, the S-curve for the plasmolysed cell continuing in a straight line, with P coincident with it.

If the diagram is used to represent the water relations of cells not immersed but in contact with air, the value of p can be determined from the equilibrium vapour tension. Here again the diagram can be extended to the right, but the extensions of the P- and S-curves will not necessarily coincide. If the wall resists collapse, S will increase faster than P. S being related to the vapour tension and so to p, the S-curve will continue straight as the reference line; the P-curve will cross it (cf. Meyer, 1938, p. 544).

SUMMARY

It is suggested that recurrent confusion and misunderstanding could be avoided by treating osmotic and suction pressures as potentials, in contrast with hydrostatic pressures set up by them.

Attention is drawn to a diagrammatic representation of the relations between these various quantities in systems including plant cells, adopted with modification by Tamiya from Sakamura.

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Plant Populations

II. The Estimation of the Number of Individuals per Unit Area of Species in Heterogeneous Plant Populations

BY

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With three Figures in the Text

I. INTRODUCTION

IT has been shown that if the individuals of a plant species are distributed at random an estimation of the density or number of individuals per quadrat may be obtained by observing only the presence or absence of the species in the quadrat (Blackman, 1935). If m is the mean number of individuals per quadrat, then the first term of the Poisson series gives the number of quadrats which do not contain the species and hence

$$m = -\log_e \frac{n_0}{N},$$

where n_0 is the number of quadrats with no individuals and N is the total number of quadrats.

However, if the population is a heterogeneous one in which the variance is significantly greater than the mean, then two or more parameters are needed to describe the distribution and consequently an estimate of the mean density can no longer be obtained from the simple equation of the first term of the series. This difficulty is encountered when the observed distribution can be fitted by Neyman's Contagious series (Neyman, 1939; Archibald, 1948). The basic hypothesis of this series is that the individuals occur in clusters which are distributed at random, the number of individuals per cluster also being a random number—an hypothesis which is in harmony with the known facts of plant propagation. However, the premisses on which Neyman's series was based were necessarily more complicated than those pertaining to plant populations, and Thomas (1949) has now evolved another form of the double Poisson series which, while it retains the original idea of contagion, expresses the ultimate series in a simpler form. It will be shown that by observing the number of quadrats with 0 and 1 individuals the parameters of this new series can be obtained approximately, and that they give a good estimation of the number of individuals per quadrat for heterogeneous populations.

Before considering the actual estimation of density a brief statement of the new series is given with an example to show how it may be fitted.

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II. THOMAS'S DOUBLE POISSON SERIES

In evolving a series for describing the distribution of plants when sampled by the quadrat method Thomas assumes that the individuals of a species are distributed in a heterogeneous manner, showing a certain degree of aggregation or clustering, and that (i) the number of clusters per quadrat are distributed at random with a mean m, and (ii) the number of units per cluster, additional to the first unit, is also a random number with parameter λ, and hence the mean number of units per cluster is $(1+\lambda)$.

The terms of the series are given by

$$P\{0\} = e^{-m}, \tag{1}$$

$$P\{k\} = \sum_{r=1}^{k} \frac{e^{-m}m^r}{r!}\left\{e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!}\right\}, \tag{2}$$

where $k = 1, 2, 3,\ldots$ gives the successive terms of the series and $\sum\limits_{r=1}^{k}$ stands for the summation of all values from $r = 1$ to $r = k$.

The values of m and λ are obtained from the first and second moments as follows:

$$\mu_1 = m(1+\lambda), \tag{3}$$

$$\mu_2 = m(1+3\lambda+\lambda^2), \tag{4}$$

where μ_1 and μ_2 are respectively equal to the mean and variance of the sample population.

A method of fitting the series to field data

The series may be conveniently described as a Double Poisson series since it is the sum of the product of the terms of two simple Poisson series $\frac{e^{-m}m^r}{r!}$ and $e^{-r\lambda}\frac{\lambda^{(k-r)}r^{(k-r)}}{(k-r)!}$. The terms of these two simple Poisson series for different values of k and r may be obtained by interpolation from Table LI for the Poisson series in 'Tables for Statisticians and Biometricians' (1945).

This method of fitting the series is shown in Table I, where data for *Carex flacca* is used. The observed and calculated frequencies are then compared by means of the χ^2 test, and the probability value, $P(\chi^2) = 0{\cdot}44$, indicates that they do not differ significantly. It has also been shown by Thomas that the series gives a good fit for data for *Armeria maritima* and *Plantago maritima* supplied by the writer.

III. THE ESTIMATION OF DENSITY FROM FIELD OBSERVATIONS

To obtain an estimate of density from this new series it is necessary to know only the number of quadrats with no individuals and the number with one individual. Then from the first two terms of the series we have

$$P\{0\} = e^{-m} \tag{5}$$

and

$$P\{1\} = me^{-(m+\lambda)}, \tag{6}$$

and the two parameters can be estimated from

$$m = -\log_e \frac{n_0}{N} \tag{7}$$

and

$$\lambda = -\log_e \frac{n_1}{mn_0}, \tag{8}$$

where n_0 is the number of quadrats with no individuals, n_1 is the number of quadrats with only one individual, and N is the total number of quadrats.

Then $m(1+\lambda)$ is the estimated mean number of individuals per quadrat.

TABLE I

Method for fitting Thomas's Series. Data for Carex flacca *(Chalk grassland, Pitstone, May 1947)*

Number of quadrats, 500; quadrat size, 20 sq. cm. $m = 1\cdot055$; $\lambda = 0\cdot3383$ (0·34 approx.)

		$e^{-r\lambda}\lambda^{(k-r)}r^{(k-r)}/(k-r)!$							
r	$\frac{e^{-m}m^r}{r!}$	k 0	1	2	3	4	5	6	λ
0	0·348625								
1	0·366933	0·712508	0·240598	0·041452	0·004859	0·000434	0·000031	0·000002	1
2	0·193535		0·496030	0·343945	0·116087	0·026662	0·004567	0·000628	2
3	0·068203			0·364378	0·367707	0·185685	0·062566	0·015826	3
4	0·018066				0·259565	0·349764	0·235977	0·106284	4
5	0·003837					0·184605	0·311809	0·263423	5
6	0·000680						0·131471	0·266617	6
7	0·000104							0·094530	7
	Product:	0·26143	0·088283	0·015209	0·001783	0·000159	0·000010	0·000007	
			0·095997	0·066563	0·022467	0·005159	0·000884	0·000121	
				0·024851	0·025078	0·012664	0·004267	0·001078	
					0·004689	0·006318	0·000426	0·001918	
						0·000708	0·001196	0·001010	
							0·000089	0·000181	
								0·000010	
	Sum:	0·26143	0·184280	0·106623	0·054017	0·025008	0·006872	0·004325	

Terms of series:

Frequency class .	0	1	2	3	4	5	6	7	8 and over
Observed . .	181	118	97	54	32	9	5	3	1
Calculated . .	174·31	130·72	92·14	53·31	27·01	12·50	3·45	2·16	4·4

$\chi^2 = 3\cdot76$. Degrees of freedom: $7-3 = 4$. $P(\chi^2) = 0\cdot44$.

In Table II and Fig. 1 the observed and calculated results for sixteen species from five different communities are compared, the observation having been made with quadrats of 20 sq. cm. taken contiguously (Archibald, 1948). The coefficient of dispersion or relative variance (ratio of variance to mean) for each species is also given. If we assume with Blackman (1942) that the distribution is not a random one when the coefficient of dispersion is greater or less than unity by $2\sqrt{\left\{\frac{2N}{(N-1)^2}\right\}}$ it will be seen that the parameters of Thomas's series when derived from the first two frequency classes of the distribution give an excellent estimate of density except in the case of *Plantago media*, *P. lanceolata*, and *Helictotrichon pubescens* where the difference between n_0 and n_1 is large. It is possible that for these three species a larger quadrat would give a better estimate of density since the ratio between n_0 and n_1 would then be reduced.

When the coefficient of dispersion is not significantly different from unity, as in the case of *Glaux maritima* and *Thymus serpyllum*, the probability of a random distribution is indicated. This is confirmed by the values for the parameters, for we get a negative value for λ, and $m(1+\lambda)$ is less than unity,

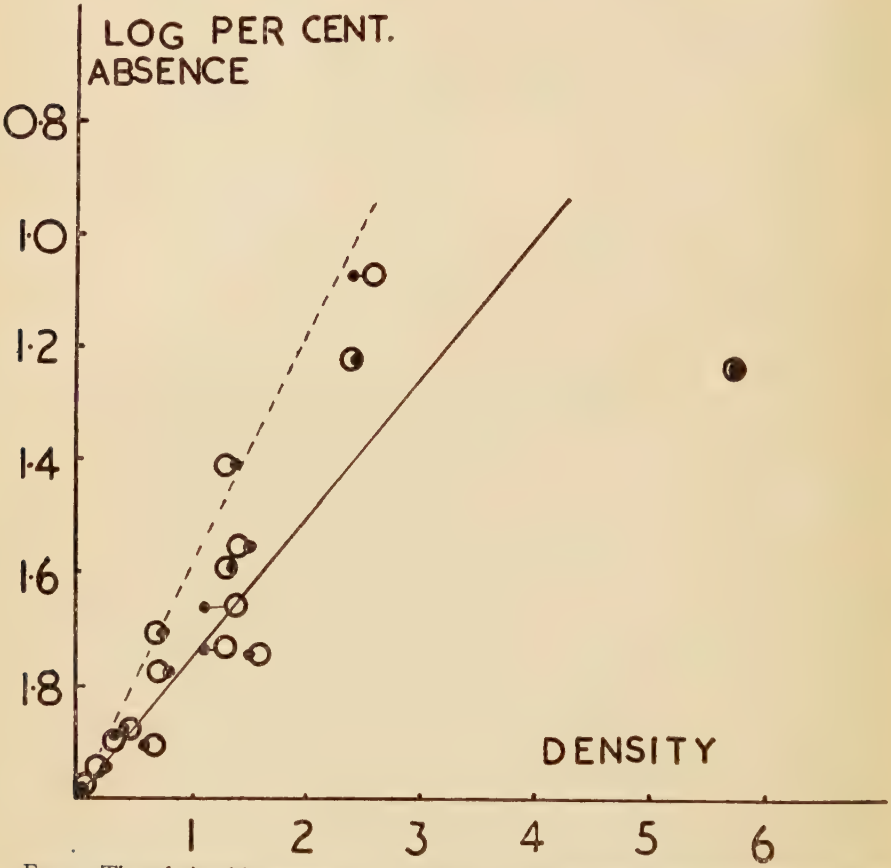

FIG. 1. The relationship between the number of individuals per quadrat (density) and the logarithm of percentage absence for different species (cf. Table II).
○ Observed density; ● density estimated according to Thomas's series. —— Regression line for estimated density according to Thomas's series coincident with regression line for field data. - - - Regression line for estimated density according to a simple Poisson series.

indicating that the double Poisson should be discarded and that a simple Poisson, value for m, would give a good estimate for density.

A comparison of the observed and estimated densities may be made if we assume with Blackman (1935, 1942) that the slope of the regression line of log percentage absence on density is given by the ratio

$$\frac{\text{mean log percentage absence}}{\text{mean density}}.$$

TABLE II

Estimation of Density (Quadrat size, 20 sq. cm.)

Species.	Total no. of quadrats.	Frequency class.		Thomas's parameters.		Density.		Coefficient of dispersion.*
		0.	1.	*m.*	$(1+\lambda)$.	Observed.	Calculated.	
Glaux maritima	500	1	15	6·214	0·119	5·796	0·738	1·107
Glaux maritima	100	26	36	1·347	0·972	1·260	1·310	0·883
Plantago coronopus	500	274	71	0·601	1·842	1·310	1·107	3·231
Carex arenaria	500	86	105	1·760	1·365	2·426	2·402	1·651
Aster tripolium	100	25	45	0·796	1·362	1·360	1·088	4·870
Plantago media	500	485	13	0·030	1·127	0·042	0·034	1·642
Plantago lanceolata	500	449	41	0·153	1·517	0·130	0·232	1·516
Helictotrichon pubescens	500	457	34	0·089	1·188	0·011	0·106	9·886
Helictotrichon pratense	500	354	58	0·345	1·745	0·658	0·602	1·941
Ranunculus bulbosus	500	368	94	0·306	1·183	0·372	0·362	0·509
Briza media	500	237	110	0·751	1·481	1·182	1·113	2·281
Carex flacca	500	181	118	1·016	1·444	1·412	1·467	1·590
Hieracium sp.	100	77	17	0·261	1·169	0·310	0·305	1·335
Briza media	100	65	18	0·431	1·212	0·610	0·522	1·603
Carex sp.	100	53	32	0·634	1·065	0·700	0·676	1·271
Thymus serpyllum	100	52	32	0·654	1·061	0·670	0·694	0·987
Festuca sp.	100	18	3	1·714	3·340	5·710	5·725	4·056
Bromus erectus	100	12	22	2·120	1·136	2·610	2·410	1·360

* Coefficient of dispersion is significantly different from unity for 500 quadrats when $>$ or $<$ $1 \pm 0{\cdot}126$ and for 100 quadrats when $>$ or $<$ $1 \pm 0{\cdot}281$.

Using this method the results for the observed and calculated densities in Tables II and V are compared in Figs. 1 and 2. The values from which the regression lines are obtained are given in Table III.

TABLE III

Regression of Log Percentage Absence on Density

Information.	No. of species.	Quadrat size, sq. cm.	Mean log % absence.	Mean density.		Mean parameters Thomas's series.	
				Observed.	Calculated.	*m.*	$(1+\lambda)$.
Table II	17	20	1·669	1·222	1·184	0·765	1·424
Table V	12	20	1·640	1·610	1·679	0·828	1·600
Table V	12	25	1·491	3·226	2·569	1·117	1·904

It will be seen that Thomas's series gives a good estimate for the density of the species in Table II whereas the simple Poisson series, for which the mean is given by the parameter *m* in Thomas's series, shows a considerable error. For the species in Table V there is a close agreement between the observed and estimated densities when a quadrat of 20 sq. cm. is used, but it is not so good for a quadrat of 25 sq. cm. These results are analysed more fully in a subsequent section when the values of the parameters *m* and $(1+\lambda)$ for different quadrat sizes are compared. It is, however, to be expected that the slope of the regression line should be affected by the quadrat size and that an increase in quadrat size will be accompanied by an increase in error of the estimation of density. In other words, the agreement between the observed and calculated results can only be expected for a limited arc of the regression line.

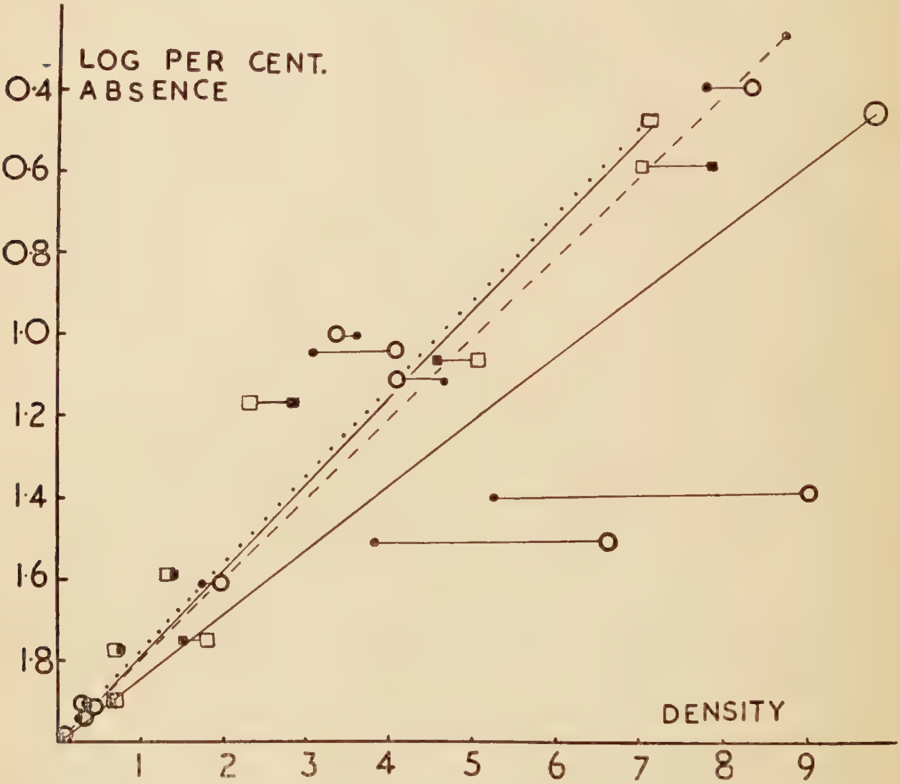

Fig. 2. Comparison of observed and estimated values for the number of individuals per quadrat of different species for two sizes of quadrat. Results indicate that a better estimate is obtained with the smaller quadrat (cf. Table V).

20 sq. cm. quadrats

□ Observed density.
□— Regression line.
■ Estimated density (Thomas's series).
. . . Regression line.

25 sq. cm. quadrats

○ Observed density.
○— Regression line.
● Estimated density (Thomas's series).
●-- Regression line.

Standard errors of estimated values

When the parameters m and λ are obtained from the first and second moments of the distribution the mean, $m(1+\lambda)$, is equal to the sample mean since the first and second moments are respectively equal to the mean and variance of the sample distribution. When, however, the parameters are estimated from the first and second frequency classes of the distribution they will be subject to error and the estimated density will also be subject to error. This is illustrated in Table IV, where the results given by the two methods are compared.

TABLE IV

Species.	Parameters from μ_1 and μ_2. m.	$(1+\lambda)$.	Density.	Parameters estimated from 0's and 1's.		Estimated density.	Observed density.
Briza media	0·6465	1·8284	1·182	0·751	1·484	1·127	1·182
Carex flacca	1·0551	1·3383	1·412	1·016	1·444	1·467	1·412
Armeria maritima	0·5735	2·7549	1·580	0·562	2·675	1·504	1·580
Triglochin maritimum	1·0300	1·2808	1·320	0·941	1·468	1·382	1·320

The method for determining the standard errors of the estimated parameters and the estimated density when derived from the first and second frequency classes of the distribution is given by Thomas (1949). She also gives tables for these quantities which are reproduced for the convenience of botanists in the Appendix of the present paper, their use being illustrated by reference to examples from plant populations.

The error in the estimation of density is partly dependent on the ratio n_0/n_1 which is in turn affected by quadrat size. From the practical point of view it is useful to have some criterion for the most suitable quadrat size to employ. A study of Tables 6 and 7 in the Appendix shows that the standard error of the sample mean and population mean increases as the ratio n_0/n_1 approaches unity. This is most likely to occur when the percentage absence is very small; in other words, when the quadrat size is such that it gives a percentage frequency of 90 to 100 per cent., the estimate of density is open to suspicion and the ratio between the first two frequency classes should be carefully examined. From the results for field observations given in Tables II and V it appears that, for a high degree of cover in grassland and herbaceous maritime communities, a quadrat of about 20 sq. cm. is suitable for estimating the density of the majority of species in the community.

IV. THE VALUES OF THE PARAMETERS IN RELATION TO QUADRAT SIZE

The parameter m has been defined as the mean number of cluster per quadrat, and $(1+\lambda)$ as the mean number of units per cluster; it is therefore of interest to both statisticians and ecologists to examine the change in the magnitude of the parameters with a change of quadrat size in order to see what the relation is between the biological cluster and the hypothetical cluster. For this purpose two sets of observations made with two different sizes of quadrat but within the same community are compared in Table V. The first series was made in September 1947 with quadrats of 20 sq. cm., and the second series in May 1948 with quadrats of 25 sq. cm.; in both series the quadrats were taken contiguously. The two series of observations were made on different patches of vegetation a few yards apart in a *Limonium* marsh community at Blakeney Point, Norfolk. In Fig. 3 the change in the parameters for nine of the most frequent species is shown graphically.

Table V

Comparison of Thomas's Parameters for Quadrats of Different Sizes

Species.	*Limonium* marsh, September 1947. 100 quadrats. Quadrat size: 20 sq. cm.							*Limonium* marsh, May 1948. 195 quadrats. Quadrat size: 25 sq. cm.						
	Frequency class.		Coeff. of dispersion.	Thomas's estimated parameters.		Density.		Frequency class.		Coeff. of dispersion.	Thomas's estimated parameters.		Density.	
	0.	1.		*m*.	$(1+\lambda)$.	Observed.	Calculated.	0.	1.		*m*.	$(1+\lambda)$.	Observed.	Calculated.
Obione portulaccoides	97	3	0·97	0·03	0·983	0·03	0·030	191	4	0·979	0·021	0·989	0·020	0·020
Spergularia marginata	97	3	0·97	0·03	0·983	0·03	0·030	192	3	0·984	0·016	1·008	0·015	0·016
Cochlearia officinalis	91	7	0·34	0·094	1·202	0·11	0·113	160	19	4·621	0·198	1·510	0·467	0·299
Puccinellia maritima	94	4	3·63	0·069	1·388	0·13	0·086	63	4	14·261	1·130	2·827	6·646	3·827
Festuca sp.	82	4	4·98	0·198	2·403	0·67	0·591	172	9	4·030	0·125	1·875	0·343	0·235
Aster tripolium	76	13	2·23	0·274	1·473	0·43	0·404	157	28	1·575	0·217	1·412	0·277	0·306
Armeria maritima	57	6	3·39	0·562	2·675	1·58	1·504	80	28	2·959	0·893	1·937	1·995	1·730
Suaeda maritima	60	18	1·50	0·511	1·532	0·68	0·783	22	32	3·278	2·182	1·406	4·117	3·068
Triglochin maritimum	39	23	1·50	0·941	1·468	1·32	1·382	26	14	2·389	2·015	2·320	4·123	4·674
Plantago maritima	12	8	2·85	2·120	2·158	5·05	4·558	49	4	10·735	1·379	3·827	9·041	5·276
Limonium vulgare	15	17	1·147	1·897	1·515	2·31	2·863	21	25	1·906	2·228	1·627	3·385	3·625
Salicornia stricta	4	3	3·90	3·219	2·428	6·99	7·814	5	6	4·527	3·664	2·116	8·282	7·753

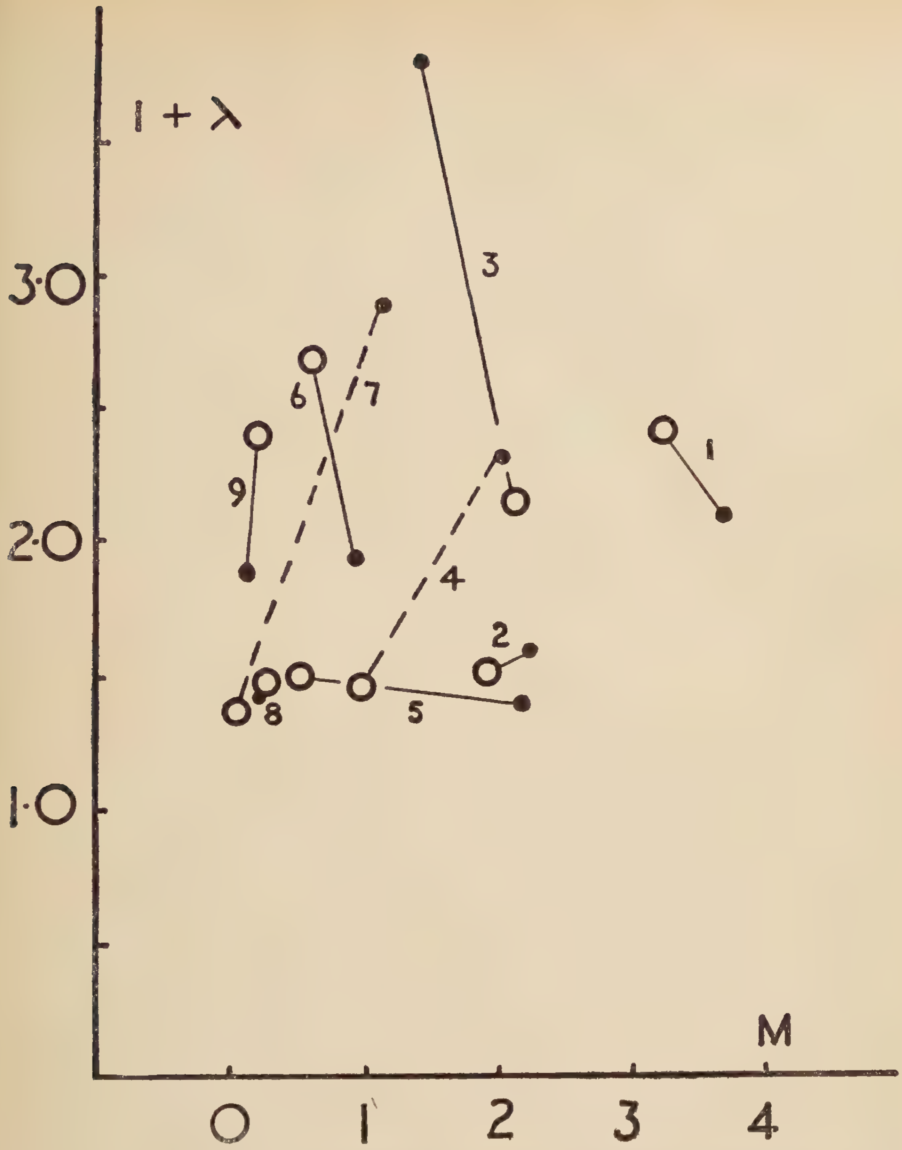

FIG. 3. Comparison of the estimated parameters of Thomas's series for pairs of similar species when observed with quadrats of different sizes.

○ = 20 sq. cm. quadrat. ● = 25 sq. cm. quadrat. m = estimated mean number of clusters per quadrat. $(1+\lambda)$ = estimated mean number of individuals per cluster.

1. *Salicornia stricta.*
2. *Limonium vulgare.*
3. *Plantago maritima.*
4. *Triglochin maritimum.*
5. *Suaeda maritima.*
6. *Armeria maritima.*
7. *Puccinellia maritima.*
8. *Aster tripolium.*
9. *Festuca* sp.

For the purpose of the present analysis the species may be considered in three sections according to the change in the absolute density. Firstly, there are the species in which the number of shoots per quadrat is less than would be expected for an increase in quadrat size of 5 sq. cm., provided the density remained constant over the whole area of the community. Under this category we have *Obione*, *Spergularia*, *Festuca*, and *Aster* and we see that, with an increase in quadrat size but a decrease in density, there is a decrease in the number of clusters per quadrat (m) while the cluster size remains more or less constant.

Secondly, there are the species in which the increase in the absolute density is almost exactly proportional to the increase in quadrat size, as in the case of *Limonium*, *Armeria*, and *Salicornia*. The parameter m shows a slight increase in all three species with the increase in quadrat size, while the parameter $(1+\lambda)$ remains more or less constant for *Limonium* and *Salicornia* but decreases for *Armeria*.

Finally, there is the group of species for which the density shows an unexpectedly great increase for an increase of 5 sq. cm. in the sample area. For *Suaeda* there is an increase in cluster number but a slight decrease in cluster size, while for *Cochlearia* and *Triglochin* there is an increase in both cluster number and cluster size. On the other hand, *Plantago* shows a decrease in cluster number but an increase in cluster size.

If we consider biological clusters, as, for example, the cluster of individuals which arise when seeds germinate in close proximity to the parent plant or the cluster of rooted shoots which grow adventitiously from a single parent, and compare them with the hypothetical clusters as defined by Thomas's series, then a possible explanation can be offered for the change in parameters with a change in quadrat size. If the hypothetical clusters were proportional to the biological clusters, and the quadrat was larger than the biological cluster then, with increase in quadrat size, we would expect an increase in the average number of clusters per quadrat (m) while the number of units per cluster $(1+\lambda)$ remained more or less constant. Except for *Plantago* this is more or less true for all species in Table V which show an increase in density with increase in quadrat size. For all these species there is an increase in the value of m, while the value of $(1+\lambda)$ is slightly above or below the expected value. If, on the other hand, the quadrat size is less than that of the biological cluster, then it is possible that the method of analysis picks out aggregation or clustering within the large natural cluster and that any increase in the size of the quadrat to include the whole natural cluster would reduce the number of clusters per quadrat but would increase the number of units per cluster. If this assumption is correct, then the change in the parameters for *Plantago* can be satisfactorily explained, and we would conclude that 20 sq. cm. was not sufficiently large to include the average *Plantago* cluster.

V. CONCLUSION

It will be seen that the present paper offers a direct mean for estimating the number of individuals per unit area for heterogeneous populations in which the variance is significantly greater than the mean. This avoids the necessity of applying a correction, as suggested by Ashby (1935), when the assumption is made that the individuals are distributed at random. Only two frequency classes are used in the estimation, and in this way the method is simpler than that suggested by Cole (1947).

The relationship between the natural clusters and the hypothetical clusters is not yet clear. In the Contagious Series Neyman defined the parameters in terms of the biological constants of the entomological problem to which his attention had been drawn, and in this ideal form the parameters are related to the spatial limits of these constants. In applying this series to plant populations it was assumed that the parameters were proportional to the number of clusters per unit area and the number of individuals per cluster. For practical purposes this is stated more simply in Thomas's series which does not attempt to define the parameters in terms of the size of the plants or of the natural clusters. Thus, while the general hypothesis of contagion appears to be acceptable, we cannot yet offer a direct relation between the parameters for clustering and the biological cluster until we know more exactly the size of the clusters of individuals for different species.

VI. SUMMARY

Data for the number of individuals per unit area for 27 species from the observation of 9,340 quadrats is discussed.

It is shown that Thomas's Double Poisson series will give a good estimate of the density of individuals in heterogeneous populations in which the variance is significantly greater than the mean. The estimate is given by the two parameters of the series which can be obtained by observation from the number of quadrats with no individuals and the number of quadrats with only one individual where

$$P\{0\} = e^{-m}$$

and

$$P\{1\} = e^{-(m+\lambda)},$$

m is the estimated mean number of clusters per quadrat and λ is the estimated number of units per cluster additional to the first unit. The estimated mean number of individuals per quadrat is equal to $m(1+\lambda)$.

Tables for the estimated parameters and the estimated mean density together with their standard errors are given in the Appendix.

The author wishes to thank Miss Marjorie Thomas of the Statistics Department, University College, London, for permission to reproduce the Tables given in the Appendix.

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APPENDIX

THOMAS'S DOUBLE POISSON SERIES

A. *The Estimation of the Parameters and their Errors*

The parameters m and λ of Thomas's series are obtained from the first and second moments of the sample distribution, or they may be estimated from the first two frequency classes of the distribution. The derivation of the estimated parameters by means of the method of maximum likelihood is given by Thomas (1949). Tables 1 to 4 give the estimated values of m and $(1+\lambda)$ and their standard errors according to this method. The appropriate equations and examples illustrating the use of the tables are given below.

Estimate of m:

$$e^{-\hat{m}} = n_0/N, \qquad \text{(Table 1)}$$

where $\hat{m}$ is the estimated mean number of clusters per quadrat,
N is the total number of quadrats,
n_0 is the number of quadrats with no individuals.

Standard error of $\hat{m}$:

$$\sigma^2_{\hat{m}} = \frac{1}{N}\left(\frac{1-e^{-m}}{e^{-m}}\right)$$

$$= \frac{1}{N}\left(\frac{1}{n_0/N}-1\right). \qquad \text{(Table 1)}$$

Estimate of $(1+\lambda)$:

$$\hat{m}e^{-\hat{\lambda}} = n_1/n_0, \qquad \text{(Table 2)}$$

where $(1+\hat{\lambda})$ is the estimated mean number of individuals per cluster, and n_1 is the number of quadrats with only one individual.

Estimate of standard error of $\hat{\lambda}$:

$$\sigma^2_{\hat{\lambda}} = \frac{1}{N}\left[\frac{1}{n_1/N}-\frac{1}{\hat{m}^2}+\frac{(1-\hat{m})^2}{\hat{m}^2 n_0/N}\right]. \qquad \text{(Table 3)}$$

The tables are calculated for $N = 100$ quadrats. The estimates of m and $(1+\lambda)$ are true for any number of quadrats, but the estimates of their standard errors are not. The error for any number of quadrats N' is obtained by multiplying the tabulated values by $10/\sqrt{N'}$.

The use of the tables is illustrated in the following example.

For *Armeria maritima* $N = 100$; $n_0 = 57$; $n_1 = 6$.

By interpolation from Table 1.

When $n_0/N =$ 0·57, then $\hat{m} =$ 0·565 and $\sigma_{\hat{m}} =$ 0·087 or an error of 15·4 per cent.

From Tables 2, 3, and 4.

When $n_0/N =$ 0·57 and $n_1/N =$ 0·06, then $(1+\lambda) =$ 2·612; $\sigma_{\hat{\lambda}} =$ 0·395 or 15·1 per cent.

TABLE 1

Estimated Values of m, $\sigma_{\hat{m}}$, and $\sigma_{\hat{m}}/\hat{m}$

n_0/N	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
$\hat{m}$	2·996	2·303	1·609	1·204	0·916	0·693	0·511	0·357	0·223	0·105
$\sigma_{\hat{m}}$	0·436	0·300	0·200	0·153	0·122	0·100	0·082	0·065	0·050	0·033
$\sigma_{\hat{m}}/\hat{m}$	0·145	0·130	0·124	0·127	0·134	0·144	0·160	0·183	0·224	0·317

TABLE 2

Estimated Values of $(1+\lambda)$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	2·097	2·527	2·862	2·977	2·992	2·936	2·814	2·609	2·272	1·637
0·10	1·404	1·834	2·169	2·284	2·299	2·243	2·120	1·916	1·579	—
0·20	—	1·141	1·476	1·591	1·605	1·550	1·427	1·223	—	—
0·30	—	—	1·070	1·186	1·200	1·144	1·022	—	—	—

TABLE 3

Estimated Values of $\sigma_{\hat{\lambda}}$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·536	0·480	0·451	0·441	0·434	0·428	0·421	0·410	0·388	0·317
0·10	0·433	0·361	0·321	0·307	0·297	0·288	0·277	0·261	0·225	—
0·20	—	0·283	0·231	0·210	0·196	0·182	0·164	0·134	—	—
0·30	—	—	0·191	0·166	0·147	0·128	0·101	—	—	—

TABLE 4

Estimated Values of $\sigma_{\hat{\lambda}}/(1+)\hat{\lambda}$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·256	0·190	0·158	0·148	0·145	0·146	0·149	0·157	0·171	0·193
0·10	0·309	0·197	0·148	0·134	0·129	0·129	0·131	0·136	0·143	—
0·20	—	0·248	0·156	0·132	0·122	0·117	0·115	0·109	—	—
0·30	—	—	0·179	0·140	0·123	0·112	0·099	—	—	—

B. *The Estimated Mean Number of Individuals per Quadrat*

In this case we wish to draw inferences respecting the total population from the observation of a number of small samples. We shall therefore distinguish between the sample mean and the theoretical population mean which is estimated from the first and second frequency classes of the sample distribution.

Values for the estimated mean $M = m(1+\lambda)$ are given in Table 8.

The standard error of the estimate of M given by the sample mean for $N = 100$ is

$$\sqrt{\{m(1+3\lambda+\lambda^2)/N\}} = \sqrt{(\mu_2/N)}, \qquad \text{(Table 6)}$$

where μ_2 is the second moment of the population.

The standard error of the estimated population mean, M, found by the maximum likelihood method, is given by

$$\sigma_M^2 = \frac{1}{N}\left[\frac{(\hat{m}-\hat{\lambda}-2)^2}{n_0/N} + \frac{\hat{m}^2}{n_1/N} - (\hat{\lambda}+2)^2\right] \qquad \text{(Table 7)}$$

The comparative reliability of the two estimates can then be judged by the ratio of the two standard errors. This is done in Table 8, which gives values for the ratio $\sqrt{\mu_2}/\sigma_M\sqrt{N}$. It will be seen from values on the left-hand side of Table 8, where n_0/n_1 approaches unity, that the ratio of the two standard errors is smallest, indicating that the maximum likelihood method gives an unreliable estimate of the population mean. Values on the right-hand side of Table 8 are high showing that the loss of accuracy due to the maximum likelihood method is small.

As an example we may consider the same data for *Armeria maritima* as was used before.

From Table 5 for the estimated mean M.

When $n_0/N = 0{\cdot}57$ and $n_1/N = 0{\cdot}06$ then $M = 1{\cdot}537$ (the observed sample mean being 1·58).

From Table 8 the ratio of the standard errors for $n_0/N = 0{\cdot}57$ and $n_1/N = 0{\cdot}06$ is 0·75, which indicates that the maximum likelihood method is satisfactory.

Table 5

Estimated Values of Mean $M = m(1+\lambda)$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	6·283	5·819	4·606	3·585	2·741	2·035	1·437	0·931	0·507	0·172
0·10	4·206	4·223	3·491	2·750	2·106	1·554	1·083	0·683	0·352	—
0·20	—	2·627	2·375	1·916	1·471	1·074	0·729	0·436	—	—
0·30	—	—	1·722	1·427	1·099	0·793	0·522	—	—	—

Table 6

Standard Error of Estimate of M given by Sample Mean for $N = 100$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·406	0·427	0·402	0·361	0·317	0·270	0·223	0·173	0·120	0·059
0·10	0·267	0·311	0·307	0·280	0·246	0·209	0·169	0·128	0·083	—
0·20	—	0·182	0·207	0·194	0·171	0·143	0·112	0·078	—	—
0·30	—	—	0·140	0·138	0·123	0·100	0·074	—	—	—

Table 7

Standard Error of Maximum Likelihood Estimate of M for $N = 100$

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	1·304	1·042	0·789	0·623	0·495	0·389	0·298	0·215	0·138	0·062
0·10	0·954	0·691	0·529	0·426	0·342	0·270	0·205	0·146	0·089	—
0·20	—	0·471	0·325	0·264	0·213	0·167	0·124	0·082	—	—
0·30	—	—	0·233	0·181	0·144	0·110	0·077	—	—	—

Table 8

Ratio of Two Standard Errors

$n_1/N \backslash n_0/N$	0·05	0·10	0·20	0·30	0·40	0·50	0·60	0·70	0·80	0·90
0·05	0·311	0·410	0·510	0·580	0·640	0·695	0·749	0·805	0·867	0·947
0·10	0·280	0·450	0·581	0·657	0·718	0·772	0·824	0·877	0·935	—
0·20	—	0·387	0·635	0·736	0·801	0·855	0·906	0·956	—	—
0·30	—	—	0·601	0·766	0·850	0·911	0·963	—	—	—

Cell-multiplication and Cell-enlargement in the Development of the Flesh of the Apple Fruit

BY

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With seven Figures in the Text

INTRODUCTION

AT maturity the apple fruit may be considered as the result of a sequence of development in which a cellular structure has been arrived at by three recognizable processes, namely, cell-division, cell-enlargement, and cell-differentiation. The main bulk of the fruit consists of a parenchymatous tissue forming the flesh, of which anatomically the cortex constitutes the greater proportion by volume.[1] The cells of the cortex comprise a spongy tissue; these cells are large, thin-walled, with a thin lining of cytoplasm and a large vacuole. The tissue is homogeneous, and the main interest in the analysis of development presented here lies in the direction of determining how cell-division and consequent multiplication of cells on the one hand, and cell-enlargement on the other hand, proceed at different stages of the ontogeny of the fruit. It can readily be seen that both the size of the fruit and the structure of its flesh will be materially affected by the periodicity and intensity of these processes.

In a previous paper (Smith, 1940) the author described the results of an investigation of the structure of the cortex of the mature apple in a number of different varieties in terms of cell-size in relation to fruit-weight. It was there shown that larger apples had both more and larger cells than smaller apples. Furthermore, the size of cell in earlier-maturing varieties was smaller, the rate of respiration higher, and the keeping quality poorer than in later-maturing varieties.

The present paper brings together the results of some studies of the course of development in the cortical region of the growing fruit in a number of varieties with widely differing characteristics, in an attempt to evaluate the parts played by the two formative processes, cell-multiplication and cell-enlargement. These studies have been extended back into the 'pre-fruit' stages of development.

[1] The percentages by weight of peel, cortex, pith, and carpellary tissues as determined for the Newton Wonder apple are approximately as follows:

Wt. of fruit in g. .	94·2	118·1	143·9	168·4	194·5
Peel . . .	5·1	5·0	4·3	4·1	4·0
Cortex . .	91·7	91·9	92·7	92·8	93·1
Pith and carpels .	3·2	3·1	3·0	3·1	2·9

[Annals of Botany, N.S. Vol. XIV, No. 53, January 1950.]

MATERIAL AND METHODS

The following varieties were selected for study:

Early Victoria (*Emneth Early*). An early-maturing culinary apple growing to relatively large size.
Beauty of Bath. An early-maturing dessert apple, attaining only relatively small size at maturity.
Worcester Pearmain. A second-early dessert apple growing to moderate size.
Lord Derby. A late mid-season culinary apple growing to large size.
Cox's Orange Pippin. A late dessert variety of small to moderate size.
Newton Wonder. A late culinary apple of moderate to large size.
Bramley's Seedling. A late culinary apple of large size.
King Edward VII. A late culinary apple of small to moderate size with characteristic late blossoming.
Court Pendu Plat. A very late dessert variety; very late blossoming. Small.

Collection of buds, blossoms, and developing fruits was made from the commencement of growth in early spring up to full maturity of the fruit, the material being fixed and stored in formalin-acetic-alcohol. The intervention of the war prevented full use being made of the material and consequently some of the data presented here are fragmentary. Such material as it was possible to examine was handled as follows. Buds were cut in half longitudinally using a sharp hollow-ground razor, washed in tap-water, and stained lightly with Delafield's haematoxylin. One-half of each bud was then mounted in glycerin on the cover-glass of a Leroux Press-chamber and examined with vertical illumination by means of the Leitz Ultrapak microscope using an immersion cap. In any one bud the king-flower was usually to be found sectioned through the floral axis together with one or more lateral flowers similarly sectioned. Using a drawing eye-piece and low-power objective, the outline of the flower and its developing organs was traced. With a higher-power objective it was possible to trace the outlines of cells lying in the cortical region between the primary vascular strands and the hypodermis; samples of 30 cells were drawn at a position approximately on a level with the middle of the developing ovary. The following measurements were recorded for 6 king-flowers and 6 lateral flowers of each lot examined (Fig. 1). The position of line *aa′* was determined by contingency with the boundary of the tissue at the base of the stamens and styles. The line *bb′* was drawn parallel to *aa′* at a distance determined by the centres of developing ovules, or before they appeared, at what was judged to be the centre of the ovary. *ab* and *a′b′* were drawn at right angles to *aa′*, *bb′*, intersecting the outline of the outer surface of the flower at a point half-way between *aa′* and *bb′*. The distance between the lines *ab* and *a′b′* (Fig. 1) was measured and designated $2r$. The distance between the lines *aa′* and *bb′* was designated l. An arbitrary rule for estimating the volume of the portion of the developing fruit which would later comprise the main bulk of fleshy tissue was to calculate the volume V of a cylinder, where $V = 2\pi r^2 l$.

When the flowers had become sufficiently developed (at the 'burst' stage[1]) they were sectioned individually, then treated in the same way. After fertilization, the fruitlets were soon sufficiently developed to cut thin sections of the cortical tissue by hand. These sections were washed, stained in Delafield's haematoxylin, and mounted on a slide in 50 per cent. glycerin in water. Drawings were made by means of a projection microscope, and the mean area of cell determined by means of a planimeter for samples of 100 cells taken from sections at opposite sides of each apple. Cell-volume was calculated on the arbitrary assumption that the actual volume was not very different from that of a sphere with equivalent cross-section in a plane through the centre. Thus, if mean area of cell $= A$, then volume $v_c = 1{\cdot}33\sqrt{(A^3/\pi)}$. In the more developed stages, owing to the gradient in size of cell between the outer and inner cortex (Smith, 1935), the precaution was taken of sampling at a point at a distance from the skin of approximately $\frac{1}{14}$th of the fruit diameter, in the equatorial plane.[2] Fruit-size was determined in the post-pollination stages by weighing, care being taken when the fruits were small to trim the calyx and pedicel.

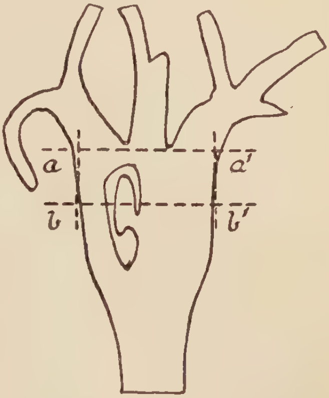

FIG. 1. Flower of the apple showing method of measurement in estimating volume during development.

DEVELOPMENT UP TO POLLINATION

Bijhouwer (1924) has traced in some detail the initiation of the floral primordia in the mixed bud and their development until early autumn, when he reported a stage of dormancy, which he attributed to prevailing low temperatures lasting until the spring. The purpose of the present investigation was to trace the subsequent development up to and including pollination and the setting of the fruit. The following recognizable stages are referred to in subsequent graphs.

Stage A. At the earliest stage examined (February 23) it was evident that growth had not yet recommenced, the buds being tight-closed. The five carpels were the least developed of the floral parts, though the cavity bounded by the bases of these carpels clearly showed the position of the future ovaries.

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[1] The terms used here to describe the stages of fruit-bud development are those used in Fruit Bud Development and illustrated by a series of charts, Bull. No. 137, Min. of Agr., H.M.S.O.

[2] Approximately 75 per cent. by volume of the fruit is contained in a shell of tissue extending from the surface to a depth of $\frac{1}{7}$th the diameter of the fruit.

Stage B. A further advance in growth of all the parts occurred prior to any external change in the appearance of the bud. The carpels began to infold at the margins as described by Kraus (1913).

Stage C. There was a further development of sepals, petals, and stamens by the time the buds began to break and the green bracts had begun to show between the scales.

Stage D. The ovules had begun to develop on the placentae formed by the infolded edges of the carpels, when the individual flowers were discernible emerging from the sheath of scales.

Stage E. By the time of the so-called 'pink-bud' stage the sepals had developed greatly and had begun to bend away from the floral axis, the styles had extended upwards, and the ovules increased in size.

Stage F. At full bloom the bases of the sepals formed a platform bearing the petals and stamens and the carpels sunk in the surrounding receptacular tissue formed the five fully developed locules.[1]

The main interest in this investigation centred round the increase in magnitude of the dimensions $2r$, l, and V as measures of the progressive development of the fleshy portion of the future fruit. In Fig. 2, therefore, is shown in a series of graphs for the Early Victoria and Bramley's Seedling varieties the values of $2r$ and l for both king and lateral flowers over the period of observation during the spring of 1939. In Fig. 3*a* and 3*b* the calculated volume V is similarly shown.

Dimension 'l'

Until the beginning of March there was scarcely any appreciable change in this dimension, but from then onwards there was an increase in magnitude that became more and more rapid until the latter part of April, when for a short period, corresponding to full bloom, enlargement ceased. It will be seen that the king flowers were of larger average dimension throughout.

Dimension '2r'

The magnitude of this dimension throughout followed the same general tendency as l.

The relation between 'l' and 'r'

It is of some interest to relate l and r for the different varieties, which are of different shape at maturity. This has been done in Table I, where the ratio l/r at the cessation of enlargement has been calculated from values extracted from the development curves and tabulated with characteristic shape of the variety, 'conical', 'tall', or 'flat'.

It will be seen from this that there is some suggestion of a tendency for

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[1] With the relative merits of the axial and appendicular theories of the origin of the apple fruit we are not concerned here. As a basis for description the terms appropriate to the axial theory are employed.

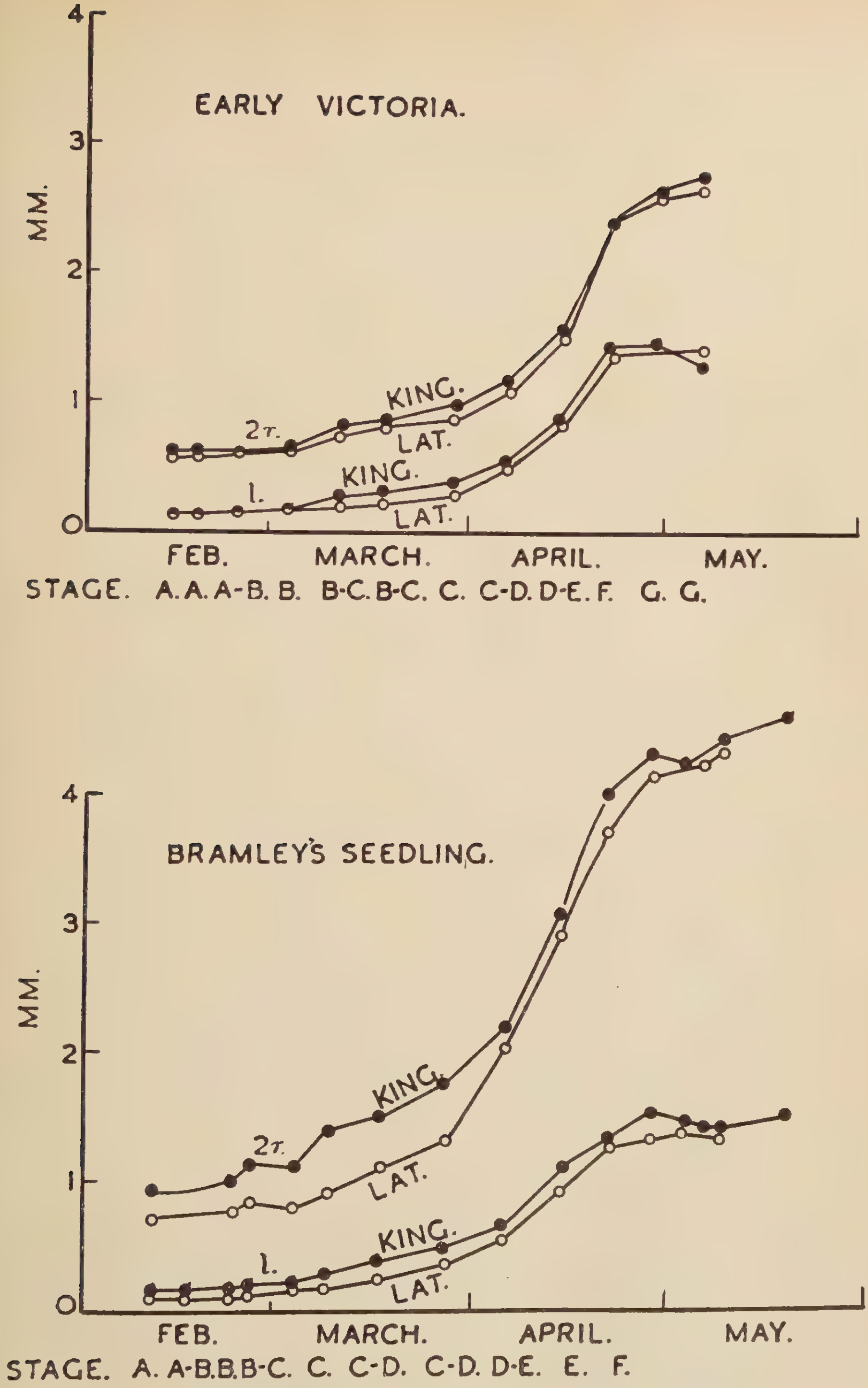

FIG. 2. Change of dimensions *l* and 2*r* during pre-pollination phase of flower development in Bramley's Seedling and Early Victoria varieties of apple. 1939.

King. = King flower. Lat. = Lateral flower.

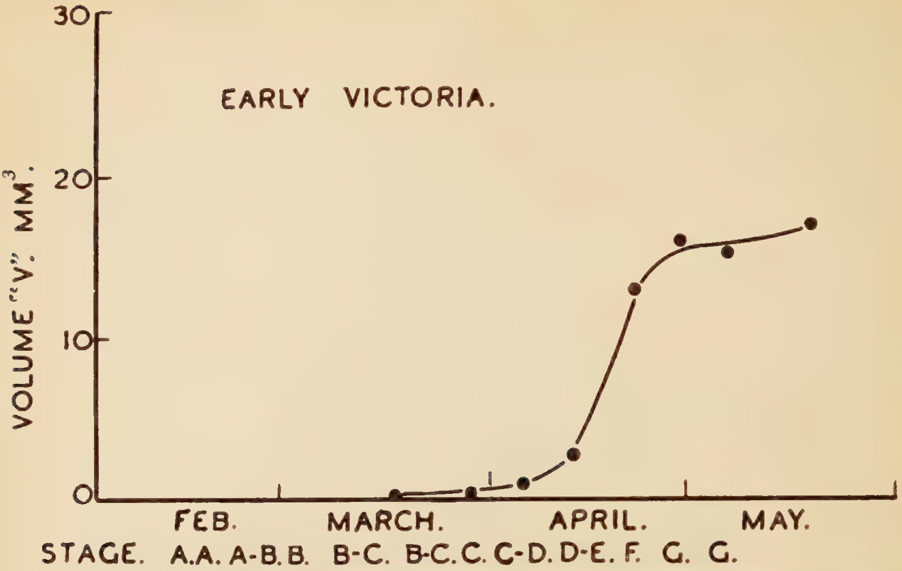

FIG. 3*a*. Changes in volume V during pre-pollination stages of development of Early Victoria variety of apple. 1939.

conical shape at maturity, as opposed to flat shape, to be foreshadowed in the flower, the flat varieties having distinctly lower values of l/r than the conical or tall varieties.

TABLE I

The Relation of the Dimensions 'l' and 'r' at Full Bloom to Varietal Shape at Maturity

Variety	l/r	Fruit-shape
Early Victoria . .	0·54	Conical
Worcester Pearmain .	0·50	Conical
Cox's Orange Pippin .	0·47	Somewhat conical
Lord Derby . .	0·46	Tall
Newton Wonder .	0·40	Somewhat flattened
King Edward VII .	0·37	Slightly conical
Beauty of Bath . .	0·37	Flat
Court Pendu Plat .	0·36	Very flat
Bramley's Seedling .	0·33	Distinctly flat

Dimension 'V'

In Fig. 3*a*, *b*, the values for V (for king and lateral flowers combined) have been plotted against time. It will be seen from these graphs that there is in general a phase of gradually increasing rate of development from the beginning of March until the latter part of April, corresponding to the time of full bloom. The curves are particularly steep during the week before full bloom, and their form suggests a quite abrupt cessation of growth in volume when the flower opens, followed again by a rapid increase, the stimulus for which may be attributed to pollination.

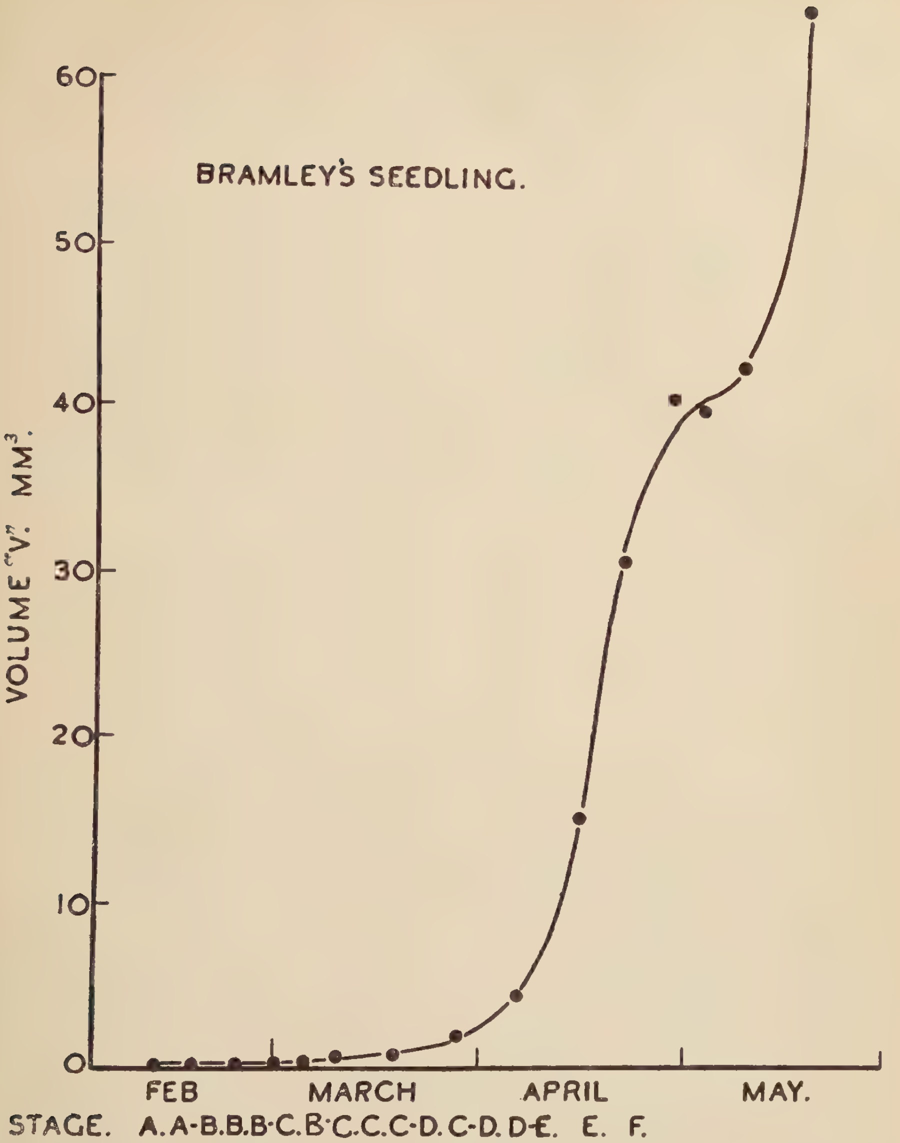

FIG. 3*b*. Changes in volume *V* during pre-pollination stages of development of Bramley's Seedling variety of apple. 1939.

CELL-MULTIPLICATION AND CELL-ENLARGEMENT BEFORE POLLINATION

The data for cell-size in the pre-fruit stages are unfortunately incomplete. The values for cell volume v_c and calculated number *N* of cells in volume *V* are plotted against time in Figs. 4 and 5 for the varieties Early Victoria and Bramley's Seedling only. It will be seen that the values are given for king and lateral flowers combined, and that for each variety there are two curves, one for volume of cell calculated as a sphere from the measured sectional area, and the other for volume of cell calculated as a cube. The actual values will lie somewhere between the two, since at this stage the cells are isodiametric and rather more cubical in shape than later when with vacuolation they become more spherical. The number N of cells per volume V has been calculated by dividing the mean volume of cell v_c into V. Here again two sets of values are plotted, the one calculated on a cubical and the other on a spherical basis.

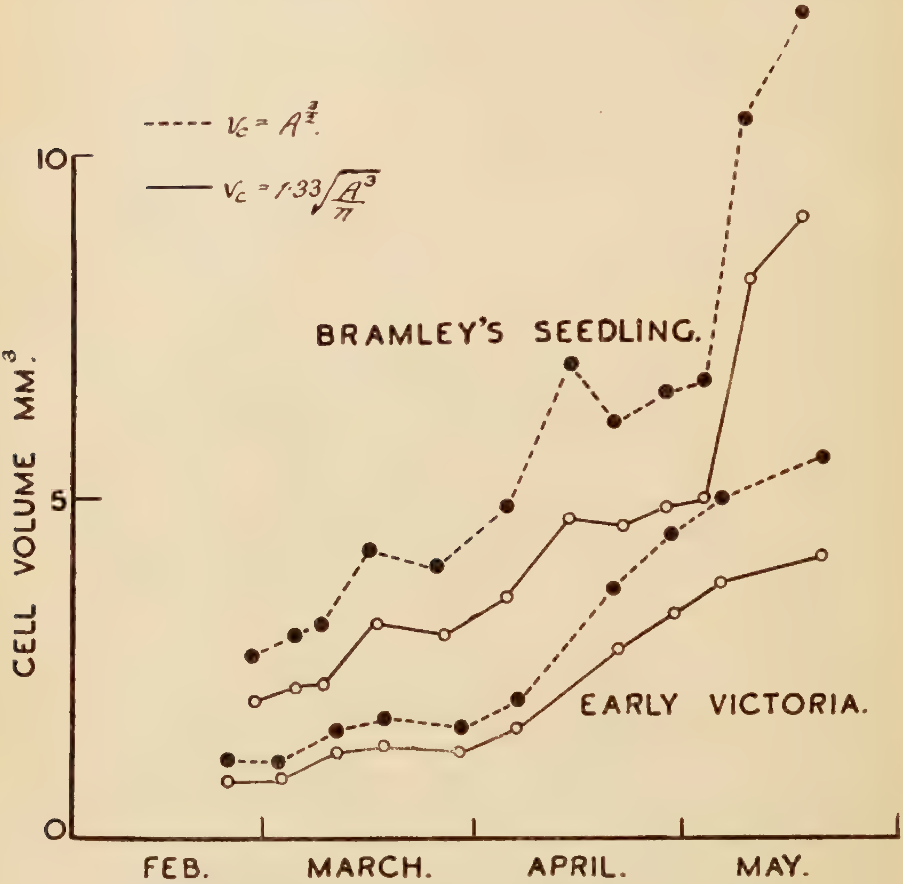

FIG. 4. Pre-pollination cell-volume v_c in Early Victoria and Bramley's Seedling varieties of apple. 1939.

It will be seen that there is a slow but steady increase in cell-volume from the earliest stages onwards up to the time of full bloom, and later a rapid increase at the time of pollination. Multiplication of cells proceeded slowly at first, but at the end of March there was a rapid increase, corresponding to the steep climb in the values of volume V. This was followed by cessation of cell-multiplication towards the end of April. (The apparent decline is doubtless due to sampling error.) During the period in which the flower is fully open there appears to be no cell-multiplication; subsequently under the stimulus of pollination there is a tremendous spurt of activity.

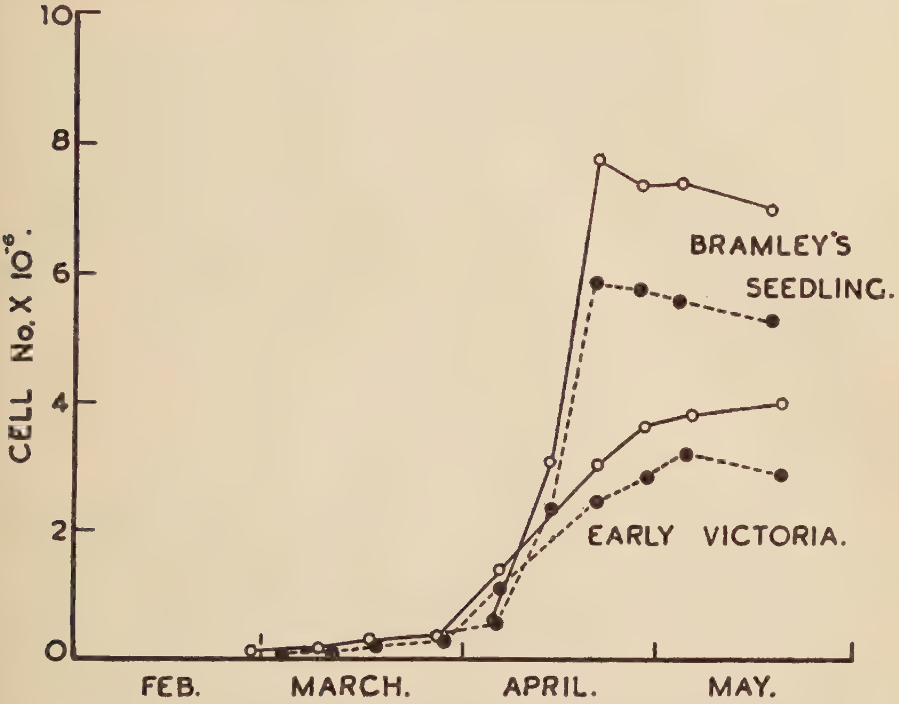

FIG. 5. Pre-pollination cell number N in Early Victoria and Bramley's Seedling varieties of apple. 1939.

The value for mean cell-volume v_c increased 4·3 times for Early Victoria and 2·5 times for Bramley's Seedling when the earliest stages examined were compared with the open-flower stage. The corresponding values for increase in number of cells were 31 times and 52 times. Thus from these observations it can be concluded that during the period of development, from the recommencement of bud-growth to the stage of fully opened flower, the dominant factor in determination of organ-size is the amount of cell-multiplication.

In Table II are shown values for organ-volume V, cell-volume v_c (mean of the values for cubical and spherical calculations), and calculated number of cells N for the nine varieties of apple examined at the stage of the fully opened flower. It will be seen that, with the exception of the varieties Lord Derby and Bramley's Seedling, these values are essentially comparable. The somewhat larger value of V for Lord Derby is attributable to a larger number of cells and of Bramley's Seedling to both greater number and larger size cells. The size of cell of the Bramley's Seedling at the commencement of bud-expansion was found to be nearly 3 times as great as that of the Early Victoria; the number of cells about 1·5 times as great. The size of the rudimentary flower was about 4 times as large as that of the Early Victoria. It is noteworthy that the Bramley's Seedling is the only triploid variety among those studied, the remainder being diploid.

TABLE II

Volume 'V', Volume of Cell 'v_c', and Number of Cells 'N' at the Stage of Fully Opened Flower

Variety.	Early Victoria.	Beauty of Bath.	Worcester Pearmain.	Lord Derby.	Cox's Orange Pippin.	Newton Wonder.	Bramley's Seedling.	King Edward VII.	Court Pendu Plat.
Volume V (mm.) .	15·0	15·0	14·7	23·8	13·1	12·3	36·1	19·3	14·6
Cell-volume v_c (mm.) . .	4·3	4·5	3·8	4·0	3·5	4·5	5·8	4·5	3·5
Cell number N ($\times 10^{-6}$) . .	3·1	3·3	3·9	6·0	3·8	2·8	6·2	4·3	4·2

DEVELOPMENT AFTER POLLINATION

With the stimulus of pollination an impulse is given for the rapid development of the fruit. The morphological and anatomical changes taking place between pollination and the completion of development at maturity have been described by others (Kraus, 1913; Tetley, 1931; Tukey and Young, 1942). Here it is proposed to deal only with those aspects that relate to the analysis of cell-multiplication and cell-enlargement as processes in the formation of the main bulk of fleshy tissue.

While the method of sampling employed precludes classification of the smoothed curves from fruit-weight plotted against time in Fig. 6 as being representative growth-curves for the crops of the nine varieties of apple during the season 1939, the general form shows the relative increase in fruit-weight as between these varieties. The main point of interest lies in the relation between fruit-weight and cell-volume during development.

From this figure and from Fig. 7, in which the mean cell-volume is also plotted against time (the origin in each graph being at May 25), it will be seen that the two sets of curves are essentially similar in form, there being in each a phase of rapidly increasing rate of enlargement followed by a phase of more uniform rate, followed again in most varieties by a fall of rate with approaching maturity.

From the values for fruit-weight and mean cell-volume, the number of cells per apple can be estimated by the formula $N = \frac{W \times 10^3}{1\cdot 1 \times v_c}$, where W is mean weight of fruit in grammes; v_c mean cell-volume in mm.^{3}, and 1·1 the value for specific gravity of the cell as calculated from measurements of the relative volume occupied by the cells in a standard volume of tissue (Smith, 1937).

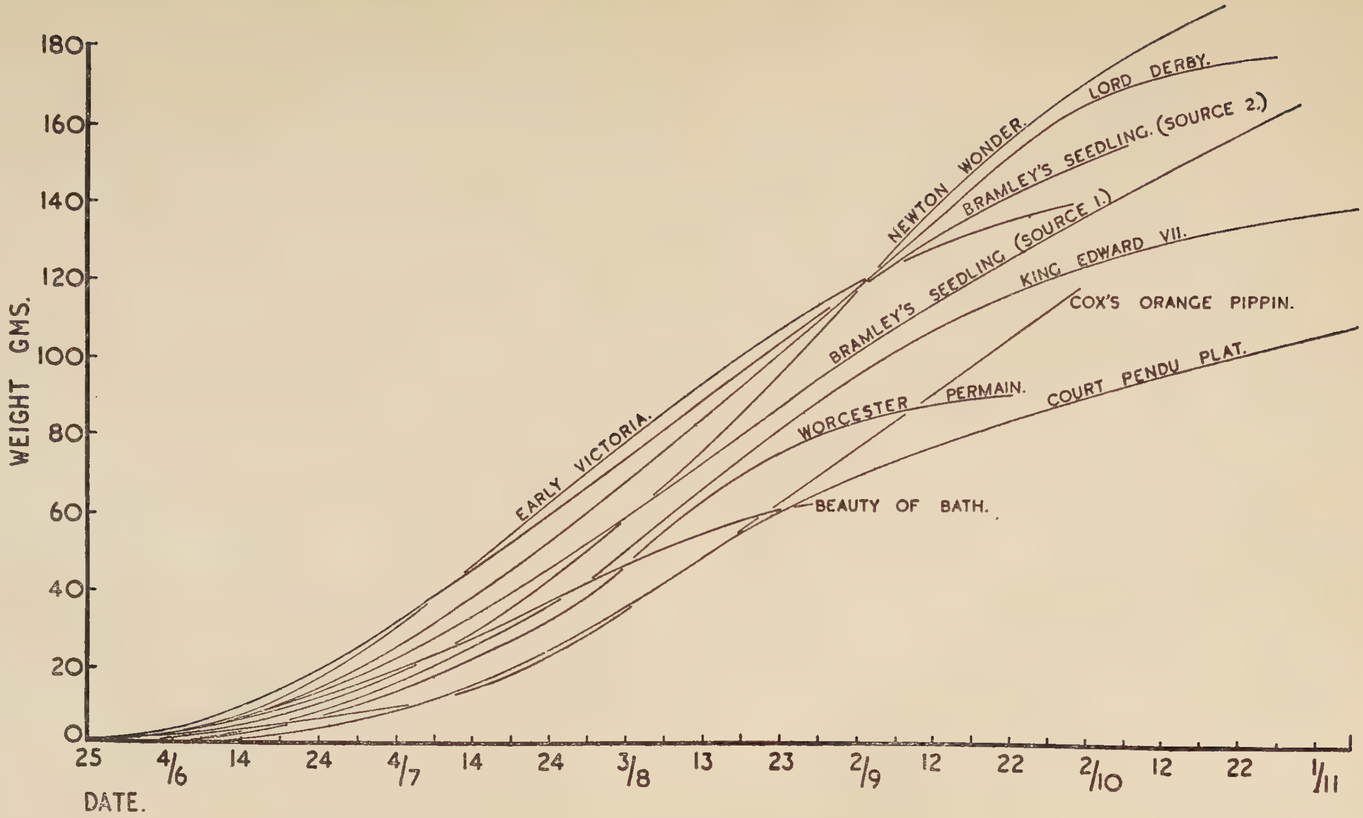

FIG. 6. Increase of fruit-weight during development of varieties of apple. 1939.

The values in Table III show that cell-multiplication is virtually completed by about the end of June, which is in agreement with the observations of Tetley (1931). Thereafter, increase in the volume of fruit is dependent upon increase in cell-volume together with increase in volume of the intercellular spaces.

TABLE III

Calculated Mean Number of Cells per Apple in Relation to Development

Variety.	Early Victoria.	Worcester Pearmain.			Lord Derby.	Cox's Orange Pippin.			Newton Wonder.	King Edward VII.		Bramley's Seedling (Source 1).	
Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).	Date.	Calculated mean no. cells ($\times 10^{-6}$).
31/5	29·0	1/6	36·3	31/5	37·9	13/6	30·7	8/6	37·4	3/6	18·2	25/5	13·3
6/6	36·5	7/6	56·7	5/6	58·3	27/6	36·5	15/6	44·9	8/6	31·7	3/6	26·8
13/6	41·4	21/6	52·7	26/6	60·6	11/7	33·4	19/6	49·9	15/6	45·9	16/6	38·3
20/6	43·1	28/6	59·0	24/7	72·1	25/7	39·4	29/6	56·7	13/7	43·5	30/6	33·9
27/6	51·4	26/7	56·0	21/8	73·8	8/8	37·3	27/7	57·4	10/8	46·2	28/7	34·7
11/7	53·3	24/8	53·1	26/9	69·3	14/9	34·1	10/8	61·0	18/9	45·2	25/8	41·1
8/8	57·9	8/9	51·9	18/10	67·6	28/9	35·3	17/10	65·8	10/10	48·9	4/10	43·5
										3/11	42·2		

THE DETERMINATION OF FRUIT-SIZE

It may now be asked whether the characteristic mean size of fruit for a variety is determined primarily by number of cells, by degree of enlargement of cells, or equally by both factors. The varieties Beauty of Bath, Early Victoria, Worcester Pearmain, Lord Derby, Cox's Orange Pippin, Newton Wonder, King Edward VII, Bramley's Seedling, and Court Pendu Plat constitute a series progressive in season of maturation. While cultural and seasonal conditions exert a decided influence upon average fruit-size, these varieties can be classed as follows: *Small*, Beauty of Bath, Cox's Orange Pippin, and Court Pendu Plat; *Medium*, Early Victoria, Worcester Pearmain, King Edward VII; *Large*, Lord Derby, Newton Wonder, Bramley's Seedling.

Reference to Figs. 6 and 7 will show that the relation between increase in fruit-weight and cell-enlargement is by no means the same for each variety. For instance, though Lord Derby develops a large apple, the rate of cell-enlargement in this variety is only intermediate and the final size of cell inferior. The reason for this is to be found in an analysis of the calculated mean numbers of cells per fruit of mean weight collected together and arranged in descending order of magnitude for number of cells in Table IV. It is then seen that, if for the moment Bramley's Seedling is left out of account, the varieties Beauty of Bath, Cox's Orange Pippin, and Court Pendu Plat are the three lowest in order of number of cells. These are characteristically small-fruited varieties. The medium-fruited varieties King Edward VII, Early Victoria, and Worcester Pearmain are next in order of ascending

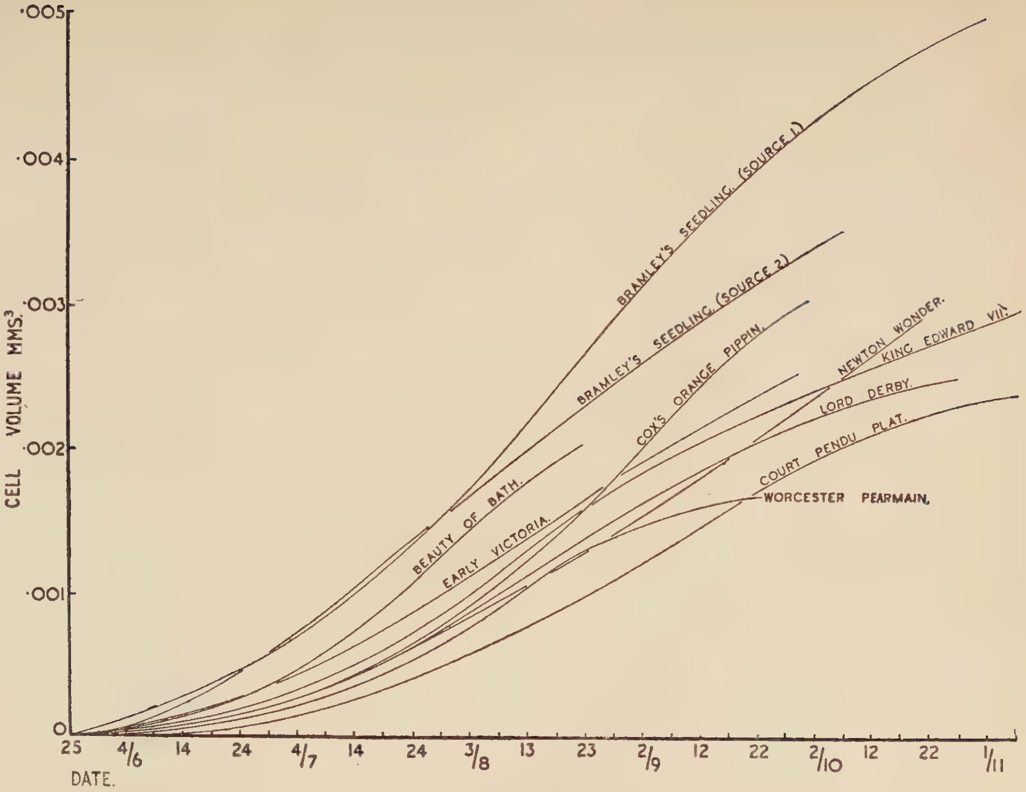

FIG. 7. Increase of mean cell-volume in flesh of fruit during development of varieties of apple. 1939.

magnitude, while Newton Wonder and Lord Derby have the largest number of cells. These two varieties are characteristically large-fruited.

TABLE IV

Mean Cell-volume and Mean Number of Cells in Mature Apple of Different Varieties

Variety.	Date.	Mean wt. (g.).	Mean cell-vol. (mm.^{3}).	Mean no. cells ($\times 10^{-6}$).
Lord Derby . . .	Oct. 25	176	0·0024	67
Newton Wonder . .	Oct. 17	200	0·0029	62
Worcester Pearmain .	Sept. 8	97	0·0016	55
Early Victoria . .	Sept. 27	142	0·0025	52
King Edward VII . .	Nov. 3	136	0·0028	44
Court Pendu Plat . .	Nov. 9	106	0·0023	42
Bramley's Seedling (Source 2) . .	Oct. 4	156	0·0037	38
Cox's Orange Pippin .	Sept. 28	116	0·0030	35
Bramley's Seedling (Source 1) . .	Oct. 27	164	0·0048	31
Beauty of Bath . .	Aug. 22	60	0·0020	27

It is again found that the triploid Bramley's Seedling variety occupies an anomalous position since it is large-fruited but with a relatively small number of cells. If, however, we exclude this variety, there is evidence that characteristic size of fruit is determined primarily by the number of cells going to form it; variation in degree of cell-enlargement is of secondary importance in this connexion.

SEASONAL VARIATION

The mean individual fruit-weight at maturity of a variety for any given tree or set of trees may be expected to vary from year to year. It will, for instance, be higher in light-crop years than in heavy-crop years. The question therefore arises: Is high fruit-weight primarily associated with a large amount of cell-division, enhanced cell-enlargement, or with both?

In Table V the mean values for fruit-weight, cell-volume, and number of cells per apple at maturity have been tabulated for apples of the varieties Bramley's Seedling (two sources) and Early Victoria. From this table it can be seen that the mean weight per fruit varied from year to year. The mean volume of cell also varied from year to year, but not in strict relation to the size of fruit. A scrutiny of the column giving number of cells per fruit of mean weight reveals also that in each series there are both high and low values. Thus in the years 1939, 1940, 1941, and 1947 the low values appear, while in 1938 and 1942 high values (Bramley's Seedling). With Early Victoria low values occur in 1938 and 1940 and high values in 1939, 1941, and 1942.

It is probable that the mean fruit-weight tends to be in inverse relation to amount of crop borne, but no records are available of total crop borne by the trees in these years. There was, however, no extreme variation.

These results suggest that mean fruit-weight may be determined in one year primarily by amount of cell-multiplication, while in another by amount of cell-enlargement.

TABLE V

Mean Cell-volume and Mean Number of Cells per Apple in relation to Mean Fruit-weight in different Years.

Date picked.	Mean fruit-weight (g.).	Mean cell-volume (mm.^{3}).	No. cells per apple of mean weight ($\times 10^{-6}$).
	Bramley's Seedling (Source 1)		
Oct. 27, 1939	164	0·0048	31
Oct. 7, 1940	177	0·0040	40
Sept. 24, 1941	130	0·0030	39
Sept. 24, 1942	170	0·0033	47
Sept. 29, 1947	132	0·0030	40
	Bramley's Seedling (Source 2)		
Oct. 3, 1938	226	0·0042	49
Oct. 4, 1939	156	0·0037	38
Oct. 7, 1940	178	0·0040	40
Sept. 19, 1941	124	0·0031	36
Sept. 24, 1942	158	0·0029	50
	Early Victoria		
Aug. 19, 1938	106	0·0020	48
Aug. 1, 1939	107	0·0016	61
Aug. 21, 1940	94	0·0020	43
Aug. 16, 1941	101	0·0016	57
Aug. 24, 1942	87	0·0013	61

CONCLUSION

The main object of the investigations in progress is to elucidate the effect of various cultural factors in the growth of the apple fruit upon its anatomical structure and the effects of variation in structure upon senescent metabolism and keeping quality. This paper is limited to a presentation of the results of the analysis of cell-multiplication and cell-enlargement during the development of the apple fruit. It is not proposed to attempt to draw inferences at this stage.

SUMMARY

An analysis was made of cell-multiplication and cell-enlargement in the development of the flesh of the fruit of nine varieties of apple, varying both in characteristic fruit-size and season. The period from the recommencement of fruit-bud development in the spring to full maturity was covered.

During the period preceding pollination, in the development of the tissues which form the basis for the future flesh of the fruit, the cells to some extent enlarged, but the main increase in bulk of tissue was due to multiplication of cells. Development ceased abruptly with the opening of the flower.

Following pollination, the rate of increase in amount of flesh and in size

of cell climbed steadily during the early phase of development. Cell-multiplication virtually ceased to have significance for the development of the flesh by the end of June, by which time a more or less steady rate of increase in weight was associated with a similar course of cell-enlargement.

The characteristic varietal size is shown to be determined primarily by the degree of cell-multiplication occurring after pollination. Varietal size was not foreshadowed in the constitution of the flower. Varietal shape was to some extent foreshadowed in the dimensions of those parts of the flower from which the fruit develops.

Seasonal variation in mean fruit-size within a given set of trees of the same variety was associated with variation in both size and number of cells in the flesh. Either or both of the factors might be determinative for fruit-size in any one particular season.

The Bramley's Seedling apple, the only triploid variety studied, was found to occupy an anomalous position in this group of varieties. In the pre-pollination stages the cells were both larger and more numerous and the volume of tissue was greater than that in other varieties. After pollination there was less cell-multiplication and more enlargement when comparison was made with other varieties.

The work described above was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research. The author wishes to acknowledge the assistance of Messrs. D. N. Rhodes, C. J. North, and W. W. Freeman.

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On the Nucleolus and Nucleolar-organizing Chromosomes of *Spirogyra*

BY

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With Plates I and II and eight Figures in the Text

INTRODUCTION

THE following is the first part of an account of mitosis in four species of *Spirogyra* in which the points established are: (1) Two nucleolar organizing chromosomes are present. (2) Two nucleoli are organized which may fuse to one. (3) The nucleoli contain coiled structures through which the greatly elongated nucleolar organizing regions of the chromosomes pass. These are designated 'organizer tracks'.

The slow dissolution of the nucleolus has been followed through prophase to metaphase. At mid-prophase a stage is reached when the nucleolus has disintegrated, but when the organizing regions of the chromosomes, contracted to approximately one-third of their length in the resting nucleolus, are still thickly surrounded by material of the organizer track. The shapes and sizes of the nucleolar organizing regions at this mid-prophase stage are somewhat characteristic for each species.

These features are of interest in that nothing has hitherto been known about the relation between chromosomes and nucleoli in *Spirogyra*; this relation, involving the presence of organizer regions of the chromosomes visibly coiled up inside the nucleolus, is unique. A modification of the aceto-carmine stain is presented as the iron-alum aceto-carmine method for algae.

The historical background of *Spirogyra* cytology consists in great part of the assertion by Van Wisselingh (1898 et seq.) that the chromosomes arose from the nucleolus, and its contradiction by Geitler (1930). The observations now made, together with the understanding of nucleolar-organizing chromosomes which has become available since 1931, has made possible a re-interpretation of the older work. The published figures of Moll (1893) and Van Wisselingh (1898 et seq.) of nucleolar structures are confirmed. The work of the above authors is discussed in the appropriate context, and references to the considerable literature will be found in their work and in that of Doraiswami (1946).

MATERIAL

The species of *Spirogyra* investigated have been identified as:

1. *S. crassa* (Kütz.) Czurda emend. 1932. Zygospores were found and the identification is certain.

[Annals of Botany, N.S. Vol. XIV, No. 53, January, 1950.]

2. *S. triformis* Van Wisselingh, 1900. Zygospores were not found. The species, not included in the Susswasserflora (Czurda, 1932), is Van Wisselingh's (1900) and is identified on the basis of the characters of the cell and of the chromosomes.
3. *S. ellipsospora* Transeau, 1914. Zygospores were not found. The species has not been previously investigated cytologically nor recorded in England. Identification is tentative on the characters of the cell.
4. *S. setiformis* (Roth.) Kützing, 1849. Zygospores were not found. The species is identified on the characters of the cell and of the chromosomes and other features of mitosis, as recorded by Geitler (1930 et seq.) and Van Wisselingh (1921). This is regarded as reasonably certain.

A table of vegetative characters (all measurements in μ) is given below.

TABLE I

	Breadth of filament.	Greatest breadth of nucleus.	Diameter of nucleolus when one only is present.	No. of chloroplasts per cell. No. of turns of chloroplast in cell.
S. crassa	150	40–6	14–16	8–10 1 turn
S. ? ellipsospora	120	25	10–12	8–10 1 turn
S. triformis	120	32–5	12	6 1 turn
S. setiformis	105	23–8	10	6 2 turns

Fixation

1. Pre-treatment. A few filaments were held in nitric acid vapour for 1 second.

2. Immediate transfer was made to 1:3 or 1:2 acetic alcohol or to Navashin with the addition of osmic acid. Occasionally (Pl. II, Fig. 1) it was first dipped in $HgCl_2$ saturated solution containing 1 per cent. chromic acid.

Species with thick mucilage sheaths do not fix well.

Staining

Iron-haematoxylin was only occasionally used since the method described below proved more satisfactory. Aceto-haematoxylin gave a good but transitory stain. Aceto-carmine as usually applied was almost useless; with ferric acetate (Thomas, 1940) it was little better.

The iron-alum aceto-carmine method for algae

Fixing times are included here. Any fixative may be used.

1. Fix till all colour is removed from chloroplasts. (*a*) in 1:3 acetic alcohol after nitric pre-treatment, about 5 minutes; (*b*) in acetic alcohol without

pre-treatment, 15 minutes or more; (*c*) in Navashin, 12 hours followed by bleaching, for osmic acid.

2. Water: few minutes.
3. 4 per cent. iron-alum solution in water: 1 or 2 seconds or more.
4. Water: few minutes.
5. Mount in aceto-carmine and heat. Blot if desired. With some species it is better not to blot.
6. Allow slide to remain warm for about 10 minutes.
7. Allow cover-glass to fall off in 95 per cent. alcohol.
8. Mount in euparal or (probably better) transfer for a few minutes to xylol and mount in balsam.

At its quickest, starting from fresh material, this schedule occupies no more than 30 minutes.

A precise and permanent stain results.

The question of artefacts consequent on the use of nitric acid vapour and heating in acetic acid may be discussed briefly. The artefacts produced, if they are to be so described, only make clear the internal organization of the nucleolus; they do not invalidate the observation of nucleolar tracks which are visible in living material examined with the Cooke phase-contrast apparatus. Nitric acid treatment is not always necessary to show them in fixed material. Van Wisselingh (1898) macerated in 50 per cent. chromic acid.

Large vacuoles seen in the nucleolus are due to poor fixation (cf. Geitler, 1930; Doraiswami, 1946) and bubble artefacts are seen in the material of the organizer track (Pl. I, Fig. 1) after over-heating.

The success of the method depends on several things which will be briefly discussed. It is thought that the stain produced is ferric carmine lake, the colour being purple-black or purple-red in the chromosomes, depending on the degree of heating and on the amount of iron present on them after the immersion in iron-alum solution and washing. It has been shown (Seki, 1933) that chromosomes strongly absorb iron from an iron-alum solution, and Gulick (1941) quotes the statement that nucleic acid has an intense affinity for Fe, less than 10^{-9} mg. giving the chromosomes a strong reaction for it. On adding aceto-carmine, the intense bluish-purple colour attributed to ferric carmine lake appears. This is soluble in hot acetic acid; thus the heating process brings about differentiation, more lake being left in the structures which had absorbed most iron. In *Spirogyra* these are the chromosomes including the organizer tracks in the nucleolus and the chromocentres.

Thus, failure of the stain may result from the iron-alum solution being too weak or the time in it too short. Overstaining and lack of differentiation result from too strong a solution of the alum, too long immersion in it, insufficient washing, insufficient heating, dilution of the acid. These things are mentioned because the material may vary somewhat in its requirements and in addition they suggest the ways of improving an overstained preparation.

It may be noted that the success of the method is at variance with the statement of Baker (1945) that the ferric carmine lake is not formed in an acid solution.

For filamentous or thin structures such as are many green algae the schedule given has many advantages; viz. (1) the colour is removed from the chloroplasts without the necessity of bleaching; (2) starch is removed in solution by the heating process. Boiling does not greatly harm the preparations. Mucilage is similarly removed or partly removed.

Thus obstacles to the view of the nucleus are got rid of incidentally to the fixing and staining processes. Other advantages are common to all methods involving heating under a cover-glass, for example, the flattening of the material. This is specially important for filamentous algae, whose usual collapse in xylol is only too familiar, and the avoidance of which has led many workers to rely on long mounting processes in Venetian turpentine or on embedding and sectioning.

Resting Stage

The resting stage of the nucleus in all the species of *Spirogyra* that I have examined so far agree in a unique feature, namely, the presence of well-defined structures inside the nucleolus. These are now described as organizer tracks. Van Wisselingh (1898 et seq.) called them *Nukleolusschläuche* or *Nukleolusfaden.* Usually they appear as a deeply staining coil in a paler background, or (Pl. I, Figs. 1, 2, 3) the whole nucleolus appears deeply staining, except for the course of the track, in resting nuclei.

The existence of structures in the nucleoli of *Spirogyra* was observed as long ago as 1893 by Moll and they were more fully described subsequently by Van Wisselingh (1898 and 1921), also of Gröningen. They were overlooked by Geitler (1930 et seq.) and those numerous investigators who have followed his line of interpretation. The resting nucleus of the species of *Spirogyra* referred to in this paper has one large or two smaller nucleoli; two organizer tracks (and presumably organizers) in a large nucleolus, one each in the smaller ones; one or more much smaller spheres of nucleolar appearance—these may be absent; and 'chromocentres' of varying size in a peripheral position in the nucleus.

The *nucleolus* has a firm membrane, well seen on crushing living material. Thus its contents would appear to be physiologically isolated to some extent at least from the rest of the nucleus.

The *nucleolar tracks* are usually not visible in living material unless the nuclei are to some extent crushed and with the ordinary microscope viewed with oblique lighting. The Cooke phase-contrast apparatus, however, shows them up clearly in *S. crassa* at least. With this the interior of the nucleolus is seen to contain loosely coiled threads or thread, brighter than the background, and of width about half that of the corresponding structure seen in a stained preparation.

Pre-treated with nitric acid vapour, fixed and stained, the organizer tracks are more deeply coloured than the background material of the nucleolus (Pl. I, Figs. 1, 2, 3). Often they appear hollow, owing to the material in the middle of the track staining more palely. Whether this is an artefact or not remains to be seen, since it is somewhat characteristic of the species *crassa* and *triformis*, whereas after the same treatment other species not to be fully described here have tracks of equally dense staining properties throughout. In *S.* ? *ellipsospora* (Pl. I, Fig. 3) the tracks are unstained in a heavily stained background; they are shorter and much less coiled than those of *S. crassa*. In *S. setiformis* after the treatment described the two slightly coiled tracks are in a peripheral position in the nucleolus, deeply stained in a pale background, while the centre of the nucleolus is occupied by one or more large globules (Pl. I, Fig. 2).

In contrast to the varied appearance of the track in the nucleolus of the resting nucleus is the much greater constancy of that of the organizing region of the chromosome during prophase (Pl. I, Figs. 4, 5, 6). The presence of two tracks in a nucleolus is best seen in recently divided nuclei while the nucleoli are still being organized. The tracks at first form independent coils; later they become more spread out and difficult to distinguish one from the other.

The small spheres of nucleolar appearance are undoubtedly the *Binnenkörper* and *Nebenkörper* of other authors (Czurda, 1922; Geitler, 1930). Their staining is like that of the background material of the nucleolus, and they are usually situated near this body in the cell. Not more than two per cell have previously been recorded, but three have occasionally been observed in my preparations. Probably there is no significance in the number of these bodies, and a reference to the development of nucleoli in telophase (to be described in a later paper) makes it likely that they are merely globules of nucleolar material which have failed to become organized. They do not become broken down during prophase as the organized nucleolus does and may still be seen at metaphase apparently unchanged. Thus it appears probable that in *Spirogyra* the nucleolar organizer is also, or is connected with, the agent causing nucleolar disorganization in prophase. These globules are not of constant occurrence in my material of *S. crassa*, *S. triformis*, and *S.* ? *ellipsospora*.

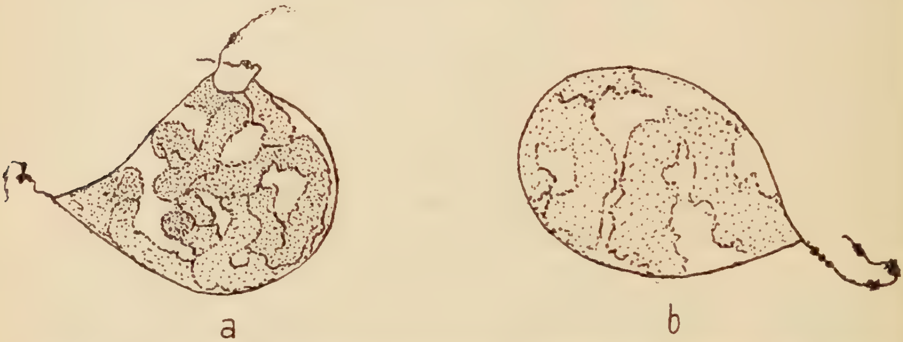
The *chromocentres*, so called following Geitler (1930), are seen as deeply stained bodies around and just within the periphery of the nucleus. In view of the difficulty of fixing chromosome structures in the resting nucleus, little significance can be attributed to their shape, which is in *S. crassa* and *S. triformis* irregular, often more or less linear, in *S. setiformis* globular. In *S.* ? *ellipsospora* they come into the category of dots and granules. Similar dots and granules are present in the other species together with the larger bodies in every gradation of size. Two points may be of interest: (*a*) their position is always peripheral; (*b*) species with large/small chromocentres have large/small chromosomes.

Geitler considered that *S. setiformis* had no chromocentres. On the other hand, he and Suematsu have stated that in certain species the number of chromosomes is the same as the number of chromocentres, and according to

Geitler the position of the chromocentres corresponds in two daughter-nuclei (Geitler, 1935*b*). It has not been possible to confirm these observations in the present investigation, and in particular *S. triformis* has approximately at least twice as many large chromocentres as chromosomes.

It may be of interest that in old filaments, whose cells will probably not divide again, of *S. crassa* and *S. triformis* the chromocentres are larger and more deeply staining than normal and the nucleoli smaller and less deeply staining.

Geitler (1935*b*), Yamaha (1935), and Suematsu (1936) have obtained a positive reaction with Feulgen's stain in the chromocentres of various species of *Spirogyra*. It may be doubted whether these so-called chromocentres are altogether comparable with those of higher plants. They presumably indicate the presence of a good deal of heterochromatin in the chromosomes of several species.



TEXT-FIG. 1. *S. crassa.* Two nucleoli from early prophase nuclei. Parts of the nucleolar-organizing chromosomes in prophase state attached at the ends of the organizer tracks in the nucleolus. The nucleolar membrane is pulled out into a point at the spot where the chromonema emerges from it.

The *chromosomes* are represented in the resting nucleus not only by the 'chromocentres'. At least in *S. crassa* and *S. triformis* after fixation, what are apparently fine closely and irregularly coiled chromonemata fill the interior of the nucleus. It might therefore be regarded as a solid nucleus in Manton's sense, although it is somewhat different from that of *Allium* (Manton, 1935). In these two species there is evidently a pronounced barrier between the nucleoli, since two nucleoli are quite commonly found, although fusion may take place during telophase, when presumably the chromosome are not yet fully expanded. These species have large chromosomes. By contrast the species ? *ellipsospora* and *setiformis* with small chromosomes practically always have but a single nucleolus in the mature nucleus, although two are always initiated at telophase. In them it is conceivable that the chromosomes are smaller or more movable structures at resting stage.

It is clear that a fine chromonema or structure representing a chromonema must extend throughout the organizer track; this chromonema is the nucleolar organizer. A theoretical diagram of the resting nucleus may therefore be constructed (Text-fig. 6, *a*) applicable to the species named in this paper, and based specially on *S. crassa*.

PROPHASE

The prophase is characterized by the long-drawn-out breakdown of the nucleolus, and the gradual emergence in a striking form of the nucleolar-organizing regions of the two nucleolar-organizing (N.O.) chromosomes (Pl. I, Figs. 4, 5, 6). The structure of the prophase chromosomes themselves will not be considered at this time.

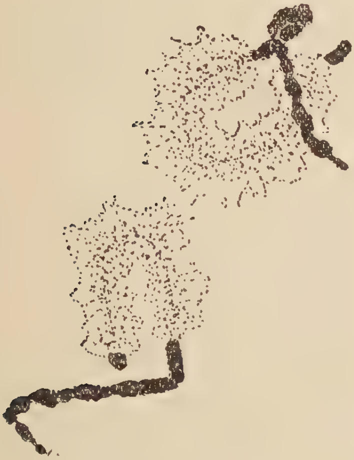


FIG. 2

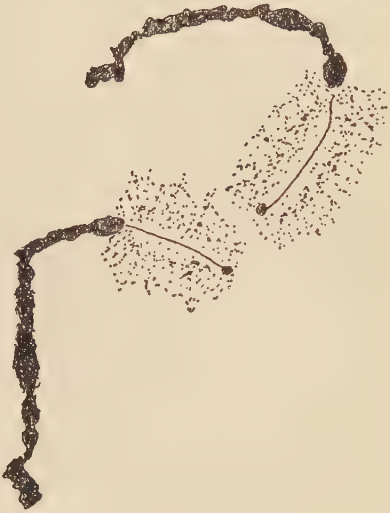


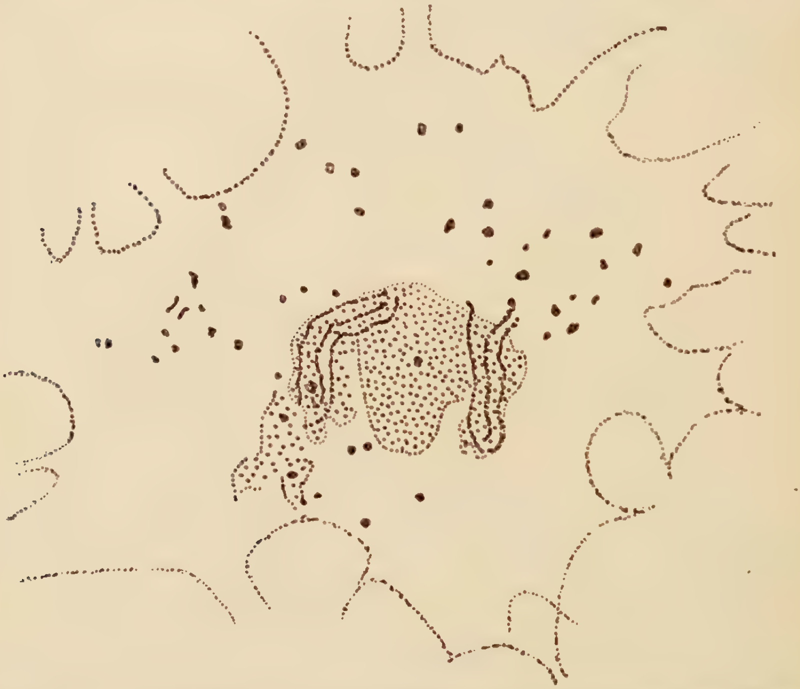
FIG. 3

TEXT-FIG. 2. *S. crassa.* Mid-prophase; two nucleolar-organizing chromosomes like Pl. I, Fig. 4. The N.O. regions are horseshoe-shaped and sub-terminal, the 'satellite' large.

TEXT-FIG. 3. *S. triformis.* Mid-prophase, the two nucleolar-organizing chromosomes on Pl. I, Fig. 5; Pl. II, Figs. 1 and 2. The N.O. regions are sub-terminal, the 'satellite' very small. The regions are straight.

In *S. crassa* and *S. triformis* there is first of all an increase in size of the nucleolus as a whole, together with a contraction in length but increase in width of the organizer tracks. They gradually uncoil and eventually assume a horseshoe shape, the two being clearly distinct from one another when both are included in the same nucleolus. The material composing the track becomes more diffuse in appearance and does not stain so deeply. At this stage it is often possible to see the connexion between the end of the track and the attached portion of chromosome, the nucleolus being pulled out into a small projection surface at the point of connexion (Text-figs. 1, 7). This pulling out is probably caused during the process of flattening the thread under the cover-glass. The maximum number of such connexions to the parts of the

N.O. chromosomes that should be observable is four. Three have actually been observed in one nucleolus—reasonable confirmation of the positions of the chromonemata in relation to the organizer tracks shown in Text-fig. 6, *a*. Van Wisselingh observed the attachment of the long arms of the chromosomes to the nucleolus (Text-fig. 7), but not that of the short distal region or satellite. As prophase proceeds the nucleolus loses its firm outline and the material composing it slowly disperses. Finally the organizers with the track material round them are separated and free in the nucleus; they are further contracted and the dispersal of the track material is also going on. They are now obviously part of the N.O. chromosomes (Pl. I, Figs. 4, 5, 6).

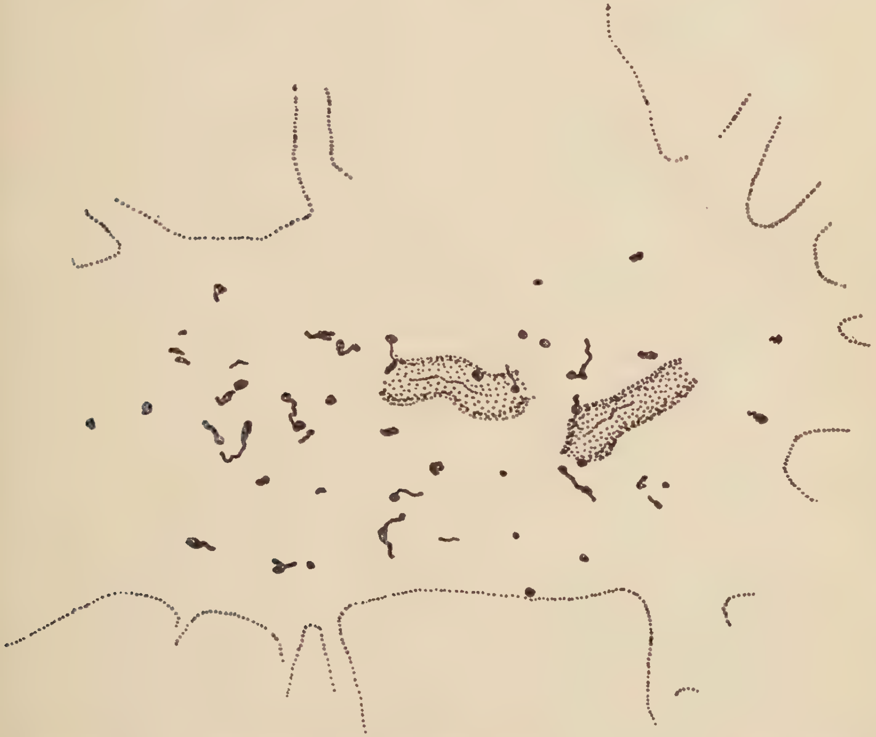


TEXT-FIG. 4. *S.* ? *ellipsospora*. Prophase, slightly earlier than mid-prophase. The two nucleolar-organizing regions are partly freed from the mass of dispersing nucleolar material and the nucleolar membrane has disappeared. Each organizer in the form of a chromonema lies in an apparent tube of deeply stained material, the organizer track material. Other chromosomes are minute.

The organizer at this stage appears as a fine chromonema, palely staining, subterminal in position. The terminal portion of chromosome is small in *S. triformis*, larger in *S. crassa*. Around the organizer lies the track material, at this stage striated by lines radiating from the organizer, and having a woolly outline. It has the appearance often associated with heterochromatin. Thus *mid-prophase* has been reached, a stage characterized in *Spirogyra* by the presence of the two organizers in a partly expanded state.

The gradual way in which this stage has been reached during the breakdown of the nucleolus is the proof of the structure of the nucleolus in the resting nucleus, and its relation with the N.O. chromosomes, shown in Text-fig. 6, *a*.

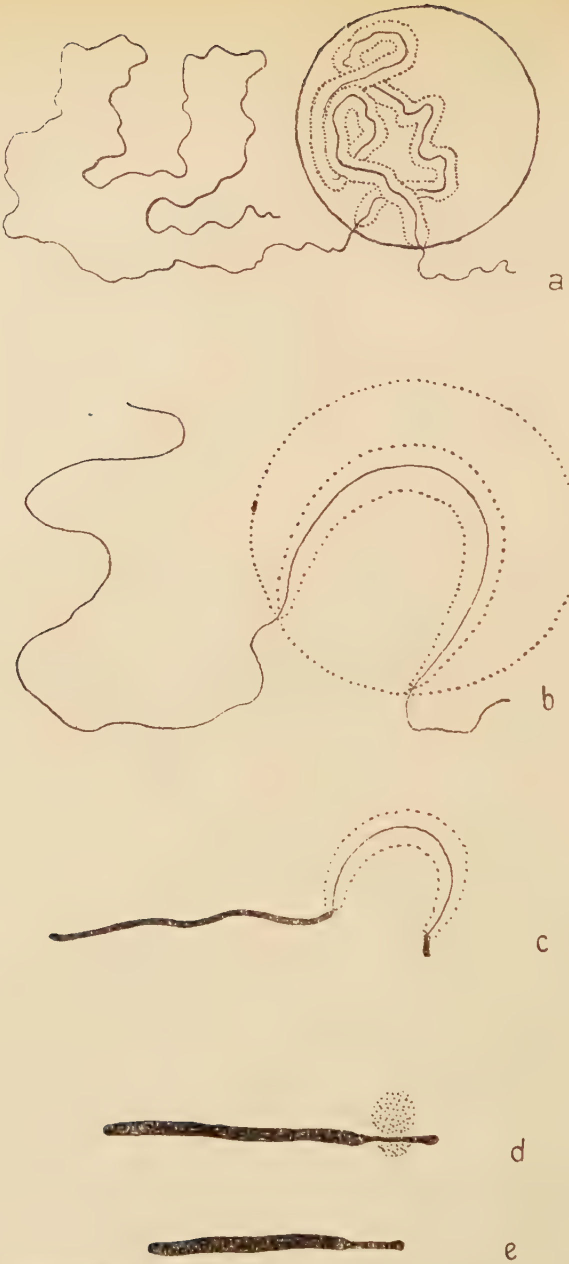
The mid-prophase of *S. triformis* is shown in Pl. I, Fig. 5, and Pl. II, Figs. 1 and 2, the same preparation in three different degrees of magnification. Pl. II, Fig. 2, shows the two N.O. chromosomes with the organizer regions; the width of the chromonema traversing the track material is somewhat exaggerated in the photograph owing to the limitations of the optical system (cf. Text-fig. 3). The small satellite is scarcely visible.



TEXT-FIG. 5. *S.* ? *ellipsospora*. Mid-prophase, shown in Pl. I, Fig. 6. Two nucleolar-organizing or N.O. chromosomes, consisting entirely of nucleolar-organizing region. The regions are more or less straight. Other chromosomes are minute.

In *S. crassa* at the same stage the organizers are somewhat longer and still curved (Pl. I, Fig. 4). The long arms of the N.O. chromosomes were out of focus in this photograph (cf. Text-fig. 2), but the larger 'satellites' are visible, showing the subterminal position of the organizer.

Van Wisselingh, who alone among the many investigators of *Spirogyra* found the mid-prophase stages described above (cf. Pl. I, Fig. 4 with his figure, here reproduced as Text-fig. 7), was influenced by the theory current in his time of the origin of chromosomes by fragmentation of a single 'spireme' thread.



Text-fig. 6. Diagrammatic representation of nucleolar-organizing or N.O. chromosome such as that of *S. crassa*. (*a*) At resting stage, the N.O. region coiled up inside the nucleolus; the region consists of a fine chromonema, embedded in a thick coating of deeply staining material, the organizer track. (*b*) At early prophase, the N.O. region contracted and no longer coiled, the organizer track broadened and the nucleolus as a whole expanded. (*c*) At mid-prophase, further contraction of all parts of the chromosome; the nucleolus has become dispersed; the N.O. region has a characteristic shape and appearance. The chromonema passing through the track material is thick enough to stain distinctly at this stage. (*d*) At later prophase, nearly maximum contraction; the N.O. region is now much thickened and the last remains of the track material lie round it loosely. (*e*) At metaphase; no sign of the nucleolus remains. All to the same scale except the satellite in (*a*) and (*b*) and the long chromosome arm in (*a*) which are arbitrarily represented. (See Table II, p. 50.)

Observing the attachment of the long chromosome arm to the organizer track, he concluded that the chromosome had emerged from the nucleolus. Therefore all the chromosomes must originate there.

Geitler and many others had no difficulty in demolishing this interpretation by establishing that the chromosomes originated outside the nucleolus. No attempt was made to find the 'nucleolar tubules'. Yet it may be seen from Geitler's figures of the nucleolus in resting stage of *Spirogyra X* (Geitler, 1935*a*) and mid-prophase of *S. crassa* (Geitler, 1930) that he may have had some evidence before him.

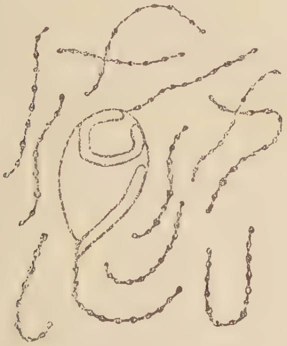


FIG. 7

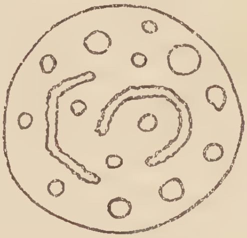


FIG. 8

TEXT-FIG. 7. *S. crassa*. Mid-prophase. (After Van Wisselingh.)

TEXT-FIG. 8. *S. setiformis*. Mid-prophase. (After Van Wisselingh, 1921.) Two curved nucleolar-organizing chromosomes consisting entirely of organizer regions, and fragments of material derived from the breakdown of the nucleolus.

Van Wisselingh's figure of the mid-prophase of *S. setiformis* is also (Text-fig. 8) reproduced; the same stage was found once in the present investigation. It shows two rather long organizers with track material and a number of lumps of other material which are found scattered about in the nucleus after the disorganization of the nucleolus.

In this species, as in the following one, no other chromosome arms or parts appear attached to the organizer, which is itself a complete chromosome and the largest of the complement. This state of affairs does not appear to have been recorded for any other organism.

In *S.* ? *ellipsospora* the breakdown of the nucleolus proceeds along lines similar to those followed in the previously described species. The organizers being less coiled from the first are practically straight, with a large mass of dispersing nucleolar material between them at an early stage (Text-fig. 4). There can be no doubts in this species as to the 'hollowness' of the organizer track (Text-fig. 4). It is clearly differentiated into a central paler staining region and a thin deeply staining outer region, the organizer itself in the form of a thin thread running through the centre of the pale staining region. It thus has the appearance of lying in a hollow tube of deeper-staining material.

At mid-prophase, the remainder of the nucleolar material having dispersed, the two organizers lie, surrounded by their track material, free in the nucleus, as in the other species (Pl. I, Fig. 6, and Text-figs. 4 and 5).

It is possible to show (see Pl. I, Figs. 4, 5, 6, all to the same scale) that the organizing regions of the N.O. chromosomes of the three species, all relatively large ones, are of the same order of size. Taking into account Van Wisselingh's figure of *S. setiformis* (Text-fig. 8), we have here four species each characterized by the shape of the organizer and other features of the N.O. chromosome at mid-prophase.

Late prophase stages show continued contraction of the organizer and dispersal of the track material. In *S. crassa* (Text-fig. 6, *d*) the last of it forms a loosely associated patch of granular material at a time when the chromosome is almost fully contracted and the organizing region itself has somewhat thickened.

In all the species the track material, and quite definitely the whole of the organized nucleolus, has dispersed when metaphase is reached.

General summary of the changes in the N.O. chromosome from resting stage to metaphase

Text-fig. 6, *a*, *b*, *c*, *d*, shows these changes diagrammatically: it is based in a general way on *S. crassa*. All the structures shown are on the same scale except the parts of the chromosome outside the nucleolus at resting stage and early prophase (*a* and *b*) which are arbitrarily represented. Only one of the two N.O. chromosomes is shown. No attempt has been made to show the structures of the chromosomes which will be described later.

The table below gives the dimensions in μ for *S. triformis*.

TABLE II

Dimensions of Nuclear Structures (*in* μ)

	Diameter of nucleus.	Diameter of nucleolus and organizer track.	Total length of N.O. chromosome.	Length of N.O. region.	Length of long chromosomes.	Length of short chromosomes.
Resting nucleus	30	12, 2·0	—	25	—	—
Early prophase	30–60	17, 3·5	41	15	26	—
Mid-prophase	—	—	25–7	8	17–18	15
Metaphase	—	—	10–12	2	8–9	7

Both diagram and table show the initial expansion of nucleolus and organizer track in early prophase. This and the striation of track material in mid-prophase are both evidence of the active chemical changes taking place. The elongation of the organizer to a length of 25 μ in the resting nucleolus is striking; since there are two such organizers, a single nucleolus may thus contain a length of some 50 μ of N.O. chromosome.

The Nature of the Organizer Track

It appeared possible to consider this a specialized part of the nucleolus, formed in proximity to the organizer, whose differential staining might be due to the fact that it is composed of substances forming a step in the synthesis of the materials composing the rest of the nucleolus. When the track appeared 'hollow' there might be two such steps, the substance next to the organizer staining little, the next layer deeply, and material outside this less deeply. On this view, the substance round the organizer (itself an independent structure having the form of a chromonema) was nucleolar material if only partly synthesized, and the name 'nucleolar tubule', a translation of Van Wisselingh's designation, was thought appropriate (Godward, 1947). I am indebted to Dr. D. G. Catcheside for the following alternative view of it: the tubule may be some special substance on the outside of the permanent part of the organizer and separating it from the nucleolar substance. In this case there would be an interface between the organizer outer substance and the nucleolar substance, at which stain behaving specially might give the appearance of a hollow tube. The contraction of the 'nucleolar tubules' goes *pari passu* with that of the satellite stalk or N.O. region. Hence they are effectively parts of the same structure; the 'nucleolar tubule' is organized by, or is part of, the nucleolar organizer rather than part of the nucleolus. There is evidence for a duality of the structure of the organizer so long as the nucleolus is present and for a short time after it vanishes. Further species of *Spirogyra* have now been found in which the organizer track is definitely not hollow and in one species has a delimiting layer of apparently membranous nature. The second interpretation therefore is preferable, and the term 'nucleolar tubule' has been abandoned in favour of the relatively non-committal name 'organizer track'.

Metaphase

A few comments only on metaphase are given here. The metaphase chromosomes of the four species described are as follows: *S. triformis*, 6 large; *S. crassa*, 12 large; *S. ? ellipsospora*, ? 70 minute; *S. setiformis*, unstainable (not counted).

The six chromosomes of *S. triformis* comprise two N.O. chromosomes, two other long chromosomes, and two short chromosomes. Since this is the haploid complement, there appears to be evidence of polyploidy even in this species. It is a curious fact too that all the species described in this paper organize two nucleoli although, as in the species *setiformis*, they may nearly always fuse in early telophase. Among other species examined two only have been found in *Spirogyra*; in other genera of Conjugales as many as four nucleoli of different sizes have been found in *Sirogonium*, two occasionally, but more often one, in *Cosmarium*, so far only one in *Zygnema*.

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HETEROCHROMATIN

Since the N.O. regions of the chromosomes are paler staining than the rest of the chromosomes at metaphase, they may be regarded as heterochromatic. It must be pointed out, however, that this result has not been obtained with Feulgen's stain, which so far has not worked with these species, in the hands of the writer. If the N.O. regions are heteropycnotic at metaphase they might be expected to be so at resting stage, where they are represented by a fine chromonema surrounded by the organizer track. The chromonema itself is certainly not stained at resting stage; its existence has to be deduced from early prophase stages, and it is in the same state as the other chromosomes. There remains the organizer-track material, which is certainly deeply stained with carmine but generally no more so than the rest of the nucleolus unless there has been pre-treatment with nitric acid vapour. Several investigators have used Feulgen's stain on various species of *Spirogyra* and obtained a positive reaction in the chromosomes. Not one of these authors (Geitler, 1935*b*; Suematsu, 1936; Doraiswami, 1946) obtained any positive reaction in the nucleolus, nor did they see any organizer tracks. It therefore seems hardly correct to regard the N.O. regions as heteropycnotic at resting stage. Positive heteropycnosis is shown at that stage by the 'chromocentres' when Feulgen's stain is used (Geitler, 1935*b*).

SUMMARY

1. The role of nucleoli in the early stages of mitosis has been followed in four species of *Spirogyra*.

2. Two nucleolar organizing (N.O.) chromosomes are present.

3. The nucleoli contain coiled structures through which greatly elongated nucleolar organizing regions of the chromosomes pass. These are designated 'organizer tracks'.

4. An iron aceto-carmine method for staining algal material is described.

CONCLUDING REMARKS AND ACKNOWLEDGEMENTS

Numerous species of *Spirogyra* have been examined in less detail than the four described. All appear to have essentially the same kind of nucleolar organization, although there appear to be considerable differences of detail. The same is true of *Zygnema* and *Closterium*. It may be too soon to assume, but it seems possible that nucleolar tracks will be found in the nucleoli of all Conjugales.

The author is especially indebted to Dr. D. G. Catcheside and Prof. I. Manton for confirmation of the observations and discussion of the manuscript; to Prof. Manton for Fig. 1, Pl. II, and also to the Central Research Fund of the University of London for the grant of £180 with which the Cooke microscope and accessories used in the investigation were purchased.

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EXPLANATION OF PLATES

Illustrating M. B. E. Godward's article, 'On the Nucleolus and Nucleolar-organizing Chromosomes of *Spirogyra*'.

PLATE I

Figs. 1, 2, 3. Nucleoli of three species of *Spirogyra* showing organizer tracks.

Fig. 1. *S. crassa* (× 2,250). Bubble artefacts in track.

Fig. 2. *S. setiformis* (× 2,330). Organizer tracks in peripheral position within nucleolar membrane; centre of nucleolus occupied by a globule.

Fig. 3. *S. ? ellipsospora* (× 2,250). Organizer tracks unstained, probably corresponding to central unstained part of organizer tracks of the other species.

Fig. 4. *S. crassa* (× 1,040). Mid-prophase, the two horseshoe-shaped bodies are the nucleolar-organizing (N.O.) regions of the N.O. chromosomes. The attached long arms of these chromosomes are not in focus. The small distal regions or 'satellites' are seen one at the upper left-hand end of the region on the left, one at the lower right-hand end of the region on the right. Parts of the other 10 chromosomes are also visible. (Cf. Text-fig. 2.) (× 1,250.)

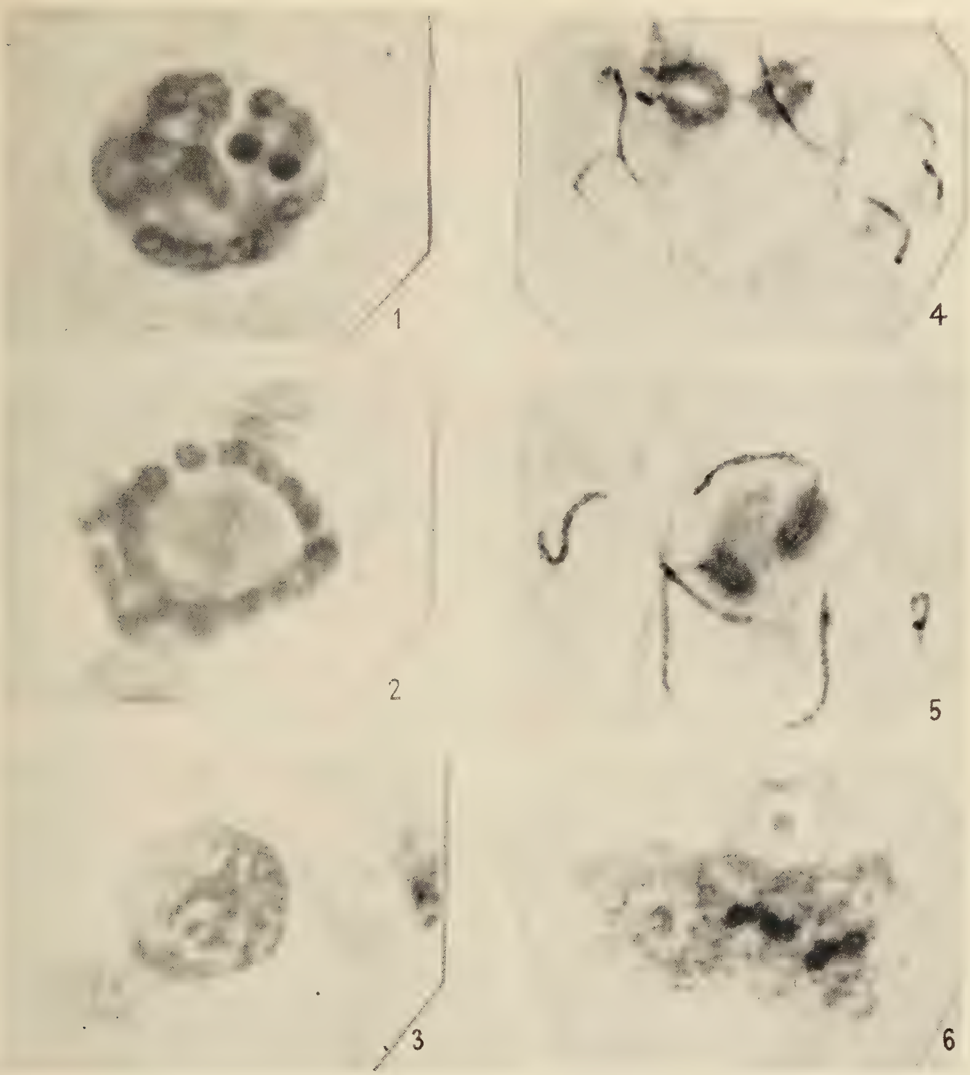
Fig. 5. *S. triformis* (× 1,040). Mid-prophase. Six chromosomes, two N.O. chromosomes, each with a straight nucleolar organizing region, to one end of which a long chromosome arm is seen attached. (Cf. Text-fig. 3.)

Fig. 6. *S. ? ellipsospora* (× 1,040). Mid-prophase. Two N.O. chromosomes consisting entirely of nucleolar-organizing region: traces of the other minute chromosomes are seen with difficulty. (Cf. Text-fig. 5.)

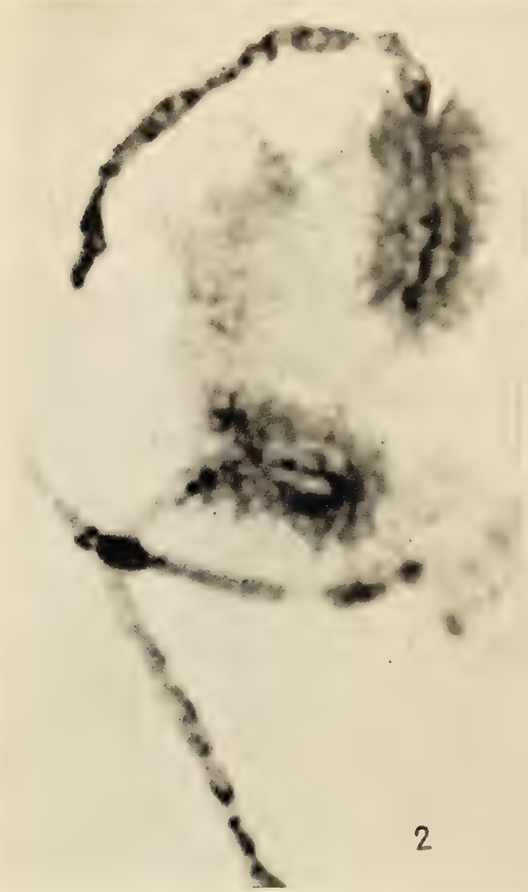
PLATE II. *Spirogyra triformis*

Fig. 1 (× 225), showing the cell with mid-prophase seen in Pl. I, Fig. 5.

Fig. 2. The nucleolar-organizing chromosomes of the cell shown above (× 2,900). The chromonema-like organizing region of the chromosomes passes through a dense mass of deeply staining material (organizer-track material). The position of the organizing region is subterminal, although the 'satellite' is very small. (Cf. Text-fig. 5.)



M. B. E. GODWARD—*Spirogyra*



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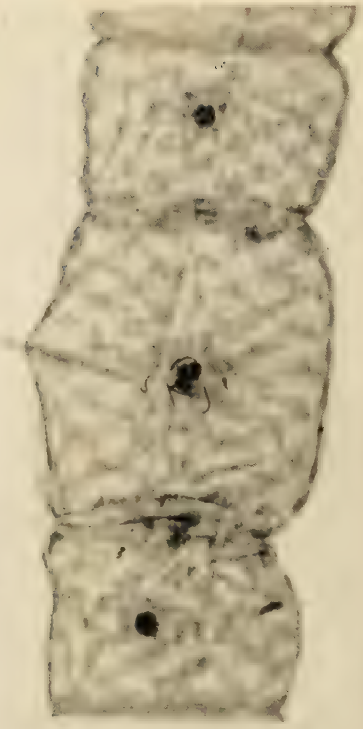

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M. B. E. GODWARD—*Spirogyra*

Physiological Studies in Plant Nutrition

XV. Assimilation of Carbon by the Ear of Barley and its Relation to the Accumulation of Dry Matter in the Grain

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With four Figures in the Text

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INTRODUCTION

THE dry weight of the ear of barley constitutes at harvest about 50 per cent. of the total dry weight of the aerial parts, and this large fraction of the dry weight is accumulated in the main over a period of 4 to 5 weeks as compared with about 10 weeks required for the growth of the vegetative organs. It is apparent, therefore, that either there must be a considerable transfer to the ear of carbohydrate synthesized during the vegetative stage of growth, as postulated in the well-known migration hypothesis, or very active assimilation must accompany the growth of the ear. In a long series of experiments dealing with sugar metabolism in barley (Archbold and Mukerjee, 1942; Archbold and Datta, 1944; Archbold, 1945) no evidence was obtained of migration on a scale which would be effective and it was concluded that the carbon needed for starch synthesis in the grain was supplied by current assimilation. During the time of rapid grain development the number of functional green leaves is rapidly diminishing, and it was long since pointed out (Déhèrain and Dupont, 1901) that the assimilatory capacity of the leaves at this time was unlikely to be adequate to supply the material for grain development. It is therefore necessary to consider the functional importance of the other green surfaces as assimilating organs and in particular of the ear itself. By shading ears of wheat (Boonstra, 1929) or barley (Watson

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and Norman, 1939; Archbold, 1942) it has been found that the dry weight of the ear is reduced by 20 to 30 per cent., and there is also evidence of the functional importance of the flag-leaf sheath (Archbold, 1942), and in oats stems have been shown to have assimilatory capacity (Stålfelt, 1935). Alternative estimates of the assimilating power of the ear were made in the experiments of Boonstra by leaving only the ears exposed to light and in those of Archbold by estimating the contribution of the ear in the absence of leaves. The values obtained varied somewhat widely from those found by simply shading the ear, indicating important effects on metabolism other than prevention of assimilation by the shaded organs. In order to obtain a valid estimate of the contribution made to its own dry weight by assimilation in the ear it is therefore necessary to measure directly the carbon dioxide uptake. Accordingly a continuous record has now been made of carbon dioxide uptake by day and emission by night by the barley ear under natural outdoor conditions, from the time of emergence until assimilation ceased with loss of all the chlorophyll. The results reported here appear to confirm the values found when ears only were shaded, rather than those found when leaves were removed in addition to ear shading, or when ears only were illuminated, in the earlier experiments. At the same time dry-weight increases and starch content of the ears has been determined for comparison with the amount of carbon dioxide assimilated.

Methods and Materials

Apparatus for measuring assimilation and respiration of barley ears

A diagram of the apparatus used for measuring carbon dioxide uptake by the ears is shown in Fig. 1. The design was adapted from that described by Chinoy (1935) and due to Professor F. G. Gregory, for measuring assimilation in leaves under outdoor conditions. The amount of carbon dioxide assimilated is measured as the difference between that absorbed from normal air and that absorbed from air which has passed over the surface whose assimilating capacity is to be determined. The essential features of the apparatus are that the gas stream used is sufficiently rapid to prevent marked depletion of the normal CO_2 of the air, while at the same time ensuring complete absorption of CO_2 after passage through the assimilation chambers, and that the gas streams in replicate circuits flow at equal rates.

Ears, attached to the plants, are enclosed in double-walled glass chambers (A), 30 cm. long and internal diameter 3·5 cm. The lower end of the chamber is left open to the air and to the upper end is sealed a glass tube (B) leading to the absorption vessel (C). Water can be circulated in the outer jacket of the chamber if desired, for cooling purposes. The absorption vessel consists of a tube 75 cm. long and diameter 2·5 cm., fitted by means of a rubber stopper to a wider tube provided below with a bulb, volume 200 ml., bearing a side arm. Below the bulb is a fine tubulure closed by a glass tap. The long tube rests upon the base of the bulb and is filled with solid glass beads to a height of 60 cm. N/1 NaOH is introduced into the bulb, and when air is flowing the liquid is raised to within 3 or 4 cm. of the top layer of beads, and the gas stream broken up into small bubbles. In this way complete absorption of CO_2 from normal air is obtained with flow rates up to 50 litres an hour. Baryta traps (N) are attached to the absorption tower, so that any failure to remove all the CO_2 may be readily detected. The tower is connected by means of a three-way tap (D) and a small needle valve (E) to a rotatory pump at (F) by means of which air is drawn through the apparatus. Six absorption towers are used in parallel connected to the pump by a common lead at (G). This lead carries a branch open to the air through a large needle valve (H). This valve is used to control the proportion of the total inflow which passes through the apparatus. Only a small part of the inflow to the pump is required to maintain appropriate flow rates in the towers, which can thus be readily adjusted.

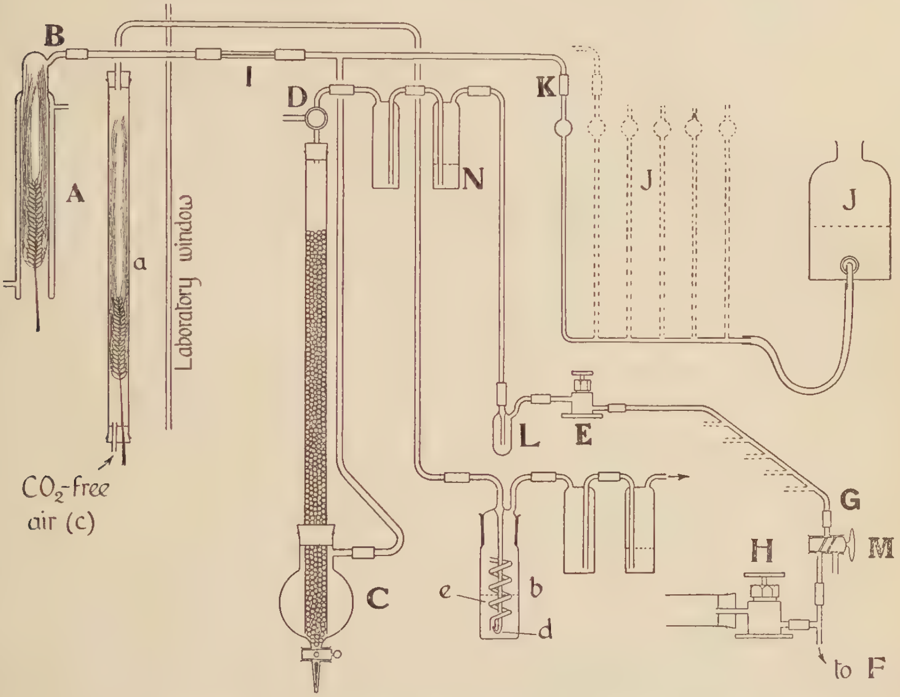

FIG. 1. Apparatus for determining assimilation and respiration by barley ears attached to the plant.

To maintain equal flow rates in the six circuits, capillary tubes about 10 cm. long and of equal resistance to flow are introduced between the assimilation chambers and the absorption towers at (I), and the pressure drop between the atmosphere and the inner end of these tubes recorded on a multiple water manometer (J). At the tops of the six manometer tubes small pieces of thermometer tubing are inserted in the rubber connexions (K) to damp out excessive oscillations. Before use, the capillary tubes are calibrated using the principle of the Wheatstone bridge, as shown in Fig. 2. Three tubes of nearly equal resistance to gas flow are placed in the arms of a bridge composed of glass tubing, at $r1$, $r2$, and $r3$. In the fourth arm is placed the tube to be tested $r4$. Across the bridge is a water manometer, and when the height of the liquid in the two manometer arms is the same, then $r1/r2 = r3/r4$ and is independent of flow rate. A number of tubes of nearly equal resistance to flow is first selected and by trial, placing each in turn in the fourth arm of the bridge, the tubes are brought to the desired value either by grinding the ends or by slightly reducing the end aperture by heating. If this latter procedure is necessary the tubes must always be used the same way round. After standardizing the capillary tubes the apparatus is assembled and the rates of flow in each circuit tested by timing the outflow from a 20-litre aspirator connected to each in turn. At this stage manometer heights corresponding to suitable flow rates are marked on squared paper fastened behind the tubes (J). At the start of an experiment the level of liquid in the manometers is adjusted as nearly as possible to the required position by first turning on the pump with the large needle valve (H) fully open to the air, and then slowly closing it until sufficient air flows through the apparatus. Final adjustments to the separate circuits are then made with the small needle valves. It is necessary to incorporate glass bulbs (L) in the leads from the absorption towers and below the level of these valves to prevent condensed water trickling into them. The common outlet tube to the pump was also provided with a tap (M) to remove water if necessary.

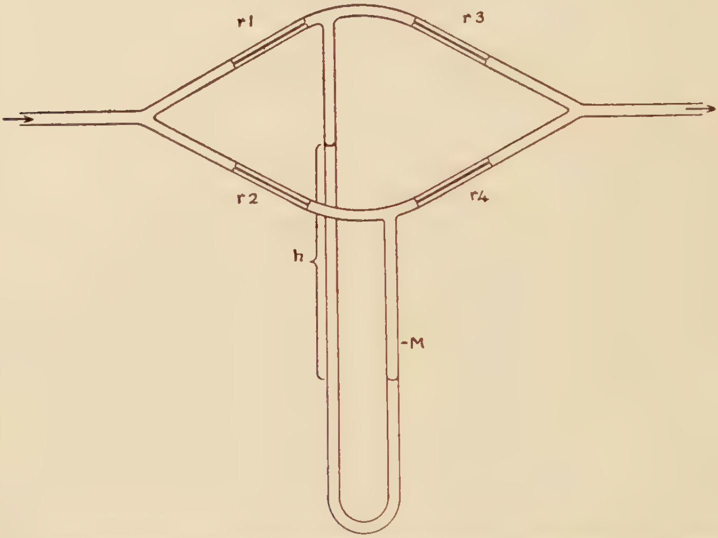

FIG. 2. Apparatus for testing resistance to air-flow of glass capillary tubes.

To conduct an experiment the bulbs and glass beads of the absorption units are first carefully cleaned and dried. The absorber is then assembled together with the three-way tap, and the side arm closed by attaching a small piece of glass rod inserted in a short length of pressure tubing. The unit is now weighed and 50 ml. of N/1 NaOH run quickly in from a burette connected to a reservoir of solution and protected from the carbon dioxide of the air. With the taps turned to exclude air the unit is again weighed and then placed in position for the experiment. When the assimilation period is completed, the three-way tap is turned off, and the side arm of the tower closed with the same glass rod and tubing, and the unit weighed to allow for any loss or gain of water during the experiment. The soda is now run out from the tower by opening the lower tap and allowing air to enter through a soda-lime tube attached to the three-way tap. It is collected in a 250-ml. flask containing 100 ml. of 10 per cent. BaCl$_2$, which precipitates the carbonate and at the same time rapidly dilutes the excess NaOH. Finally the unit is weighed once more so that the amount of NaOH run out may be determined. By this weighing technique the necessity for quantitative removal of the NaOH from the tower is avoided. After standing to allow carbonate to settle, 50 ml. of the supernatant liquid are measured out and a known volume of N/10 HCl, insufficient to neutralize all the excess NaOH, added by means of an automatic pipette. The remaining NaOH is then titrated with a further quantity of N/10 HCl and the amount of carbonate present found by subtracting the total titration value from that of an appropriate blank. A specimen calculation is shown below:

	Weight of absorption unit g.	Wt. NaOH g.
1. Empty	611·20	—
2. +50 ml. N/1 NaOH, before experiment	663·35	52·15
3. + „ „ after „	662·25	51·45
4. After running out NaOH	617·95	44·70

44·70 g. NaOH made up to 250 ml. and aliquots titrated with HCl.

ml. N/10 HCl required for 50 ml. NaOH (1) 26·43+18·30, (2) 26·43+18·25; Total 44·71.

Hence ml. N/10 HCl required for excess NaOH in 52·15 g. taken =

44·71×5×[51·45/44·70] = 257·31
ml. HCl required for blank = 469·91
Hence ml. HCl equivalent to CO$_2$ absorbed = 212·60

Duration of experiment 42 hours, hence mg. of CO$_2$ absorbed in 42 hours = 467·7 or 11·13 per hour.

After running out the NaOH the absorption units are dismantled and the beads and glass parts washed free from alkali and dried ready for reassembling.

Details of the respiration apparatus are included in Fig. 1. Ears attached to the plant are enclosed in glass tubes (*a*), diameter 2·5 cm., closed at the top with a one-holed rubber stopper, carrying a glass tube leading to the absorption bottles (*b*), and at the bottom with a two-holed stopper. This stopper is slit up the side to allow the peduncle of the ear to be introduced into one of the holes, and the joint made air-tight with a mixture of bees-wax and vaseline.

Air freed from CO_2 by passage through 30 per cent. KOH and a 2-ft. soda-lime tower at (*c*) is drawn through the glass tube inserted in the second hole of the bottom stopper, and enters the absorption bottle through a fine jet (*d*) over which rests a glass spiral (*e*). This device breaks up the gas stream and so ensures complete absorption. The CO_2 is determined in the same manner as described for the measurement of assimilation, by weighing the absorption bottles containing the NaOH, here N/2, and suitable titration of the excess remaining at the end of the experiment.

Material

Barley, Spratt Archer, was grown in the open in 10-in. glazed pots in the summer of 1946. Eight seeds per pot were sown on May 2 and complete mineral nutrients given in three equal doses on May 14, when the seeds had germinated, on May 28 as the third leaf emerged, and on June 11 as the fifth leaf emerged. The total amounts of salts added per pot were: $Na_2HPO_4.12H_2O$, 2·52 g.; K_2SO_4, 1·85 g.; $NaNO_3$, 9·0 g.; $MgSO_4.7H_2O$, 1·25 g.; $CaCl_2.6H_2O$, 0·37 g.; $FeCl_3$, 0·15 g.; $MnSO_4.4H_2O$, 0·10 g. At the time of the second dose the plants were thinned to four per pot, and at the fifth-leaf stage the main axis on each plant marked. Only ears on these main axes were used in subsequent experiments.

Assimilation measurements were made during the day and respiration measurements during the night on ears attached to the plants and subject to the natural fluctuations of light and temperature. The measurements were begun at the time of ear emergence. Three pots were set up outside the laboratory window, and an ear on the main axis of one plant from each pot introduced into an assimilation chamber; three empty chambers, to act as controls, were placed over the pots at the same height as those containing ears, and all six chambers connected up to the absorption units inside the laboratory. Air was then drawn through the chambers at 25 litres an hour, as already described, during the daylight hours of three successive days. At dusk the taps were closed to exclude air, the gas stream turned off, and the chambers lifted off the ears, and at dawn the chambers were replaced and the gas stream again started. A second set of ears from the same pots was introduced into the respiration chambers at dusk on the first day and the carbon-dioxide-free gas stream allowed to flow during the dark hours of three successive nights, at about 6 litres an hour. At the end of the 3-day period the ears were finally removed from the chambers, detached from the plants, weighed, and preserved in alcohol for dry weight and starch determinations. The assimilation and respiration absorbers were removed for estimation of the carbon dioxide absorbed, and were at once replaced by a second set. Another group of three pots was set up as quickly as possible and connected to the second set of absorbers and the gas stream started. The time taken to change the plants and absorbers was from 10 to 30 minutes. This procedure was repeated until assimilation ceased, with loss of all the chlorophyll, as the ears reached the full ripe condition. Eleven sets of observations were made, covering a period of 4½ weeks, during which ears were assimilating, together with two sets of measurements after assimilation had ceased, and three sets on the flag leaf and its sheath before ear emergence.

Dry weights were separately determined on the whole ear and on the grain, giving, by difference, the dry weight of the awns, rachis, &c. For the estimation of starch, ears preserved in alcohol were transferred to Soxhlet thimbles, and extracted with fresh alcohol overnight. The material was then dried and weighed, and the grain separated and weighed. The grain was then finely ground in a Wiley mill, and aliquots of the powder used for estimation of starch by the method of Hanes (1936). The same β-amylase preparation was used for all the determinations, and the hydrolysis limit found for a preparation of starch made from a sample of the barley used for sowing the experimental pots. Under the conditions used, namely, 24 hours' incubation at 26° C. and pH 4·8, the percentage hydrolysis was 59·5, the mean of six determinations carried out along with the estimations of starch in the experimental samples. The reducing power of the hydrolysates was determined using Somogyi's (1945) reagent recommended for maltose estimations, and the results calculated as starch.

EXPERIMENTAL RESULTS

The mean rates of carbon dioxide absorption per hour in the six circuits of the assimilation apparatus for ten 3-day periods are shown in Table I. In five cases circuits 1, 4, and 6 were connected to empty assimilation chambers and circuits 2, 3, and 5 to chambers containing ears, and in the other five cases the positions were reversed. Analysis of variance of this data was used to determine the standard errors appropriate to the assimilation values. The mean

TABLE I

Mean Hourly Rates of Carbon Dioxide Absorption in the Six Circuits of the Assimilation Apparatus

Date 1946.	Total hours.	Controls (no ears)			Assimilating (with ears)		
		1.	4.	6.	2.	3.	5.
				mg. of CO_2.			
24/7 to 27/7	46·42	11·82	11·93	11·76	10·39	10·11	10·33
30/7 „ 2/8	45·58	11·73	11·98	11·63	10·21	10·12	10·68
2/8 „ 5/8	45·58	11·98	12·07	11·98	10·87	10·28	10·35
8/8 „ 11/8	44·58	11·63	11·84	11·64	10·96	10·17	10·56
11/8 „ 14/8	43·92	11·31	11·31	11·36	10·47	10·46	10·19
		2.	3.	5.	1.	4.	6.
14/8 „ 17/8	43·83	11·69	11·23	11·44	10·13	10·83	10·07
17/8 „ 20/8	42·92	11·92	11·23	11·44	10·28	11·40	10·82
20/8 „ 23/8	42·25	12·00	11·42	11·87	11·15	11·14	11·76
23/8 „ 26/8	41·83	11·45	11·25	11·26	11·33	11·19	11·94
26/8 „ 29/8	41·17	11·21	11·69	11·07	11·53	11·44	11·38

Mean 11·62±0·09 10·78±0·09

Standard error of the means of 30 values 0·13

„ „ „ „ 3 „ 0·40

rates per hour of carbon dioxide absorption over a 30-day period in the control experiments and in the experiments in which ears were introduced into the chambers were respectively 11·62 mg. and 10·78 mg., with a standard error of 0·09 on these means of 30 estimates. The mean rate of assimilation over the whole time ears were assimilating was 1·29 mg. per ear per hour, and this figure is subject to an error of 0·13, or about 10 per cent. The mean rates of carbon dioxide emission by night for seven experiments are shown in Table II. Over a period of 21 days the rate of carbon dioxide output per hour per ear was 0·44 mg. with a standard error of 0·03, or about 7 per cent.

TABLE II

Mean Hourly Rates of Carbon Dioxide Emission by Night in the Three Respiration Circuits

Date 1946.	Total hours.	1.	2.	3.
21/7 to 24/7	24·42	0·275	0·202	0·162
24/7 „ 27/7	25·08	0·287	0·286	0·284
30/7 „ 2/8	26·33	0·764	0·841	0·562
2/8 „ 5/8	26·08	0·688	0·485	0·273
5/8 „ 8/8	26·42	0·592	0·891	0·802
14/8 „ 17/8	28·08	0·339	0·280	0·319
20/8 „ 23/8	28·25	0·261	0·188	0·165

Mean 0·426±0·025

Standard error of the means of 30 values 0·022

„ „ „ „ 3 „ 0·060

The hourly rates of assimilation and respiration for each 3-day period from the time of ear emergence until the ears were no longer green (21/7 to 23/8) are shown in Fig. 3. The mean hourly rate of assimilation remained practically constant at about 1·45±0·40 mg. of carbon dioxide from the time of ear emergence for about 18 days and then fell off to zero during the ensuing 12 to 15 days as the ears ripened and the chlorophyll disappeared. The mean hourly rate of respiration by night rose as the ears emerged to a maximum of about 0·70±0·65 mg. per ear per hour, and then fell off to a low value of about 0·20 mg. per ear per hour. In addition to these values estimates of the day-time respiration were made on two occasions, during the periods 2/8 to 5/8 and 17/8 to 23/8, by measuring the carbon dioxide emission by day of darkened ears kept otherwise under the same conditions as prevailed for the concomitant measure of assimilation. The values recorded were respectively 1·01 and 0·79 mg. per ear per hour, as compared with night respiration values of 0·48 and 0·32 mg. per ear per hour and net assimilation values of 1·50 and 0·78 mg. per ear per hour. The derived gross assimilation values of 2·51 and 1·57 mg. per ear per hour are also marked in Fig. 3. Finally the determinations made of the net assimilation rate of the flag leaf and its sheath during the 6 days preceding ear emergence and 3 days as the ear emerged are also indicated in Fig. 3 and show that over this time the amount of carbon dioxide assimilated by these organs was of the same order as that of the emerged ear.

The net uptake of carbon dioxide by the ear over the 33 days from 21/7 to 23/8 during which ears were assimilating was 622±65 mg. By calculation from the two estimates of respiration during the day the gross uptake was probably about 1,057 mg. per ear, during 496 daylight hours. The total emission of carbon dioxide by night was 117±7 mg. per ear during 296 dark hours. In terms of hexose this gives a net assimilation figure of 423 mg.

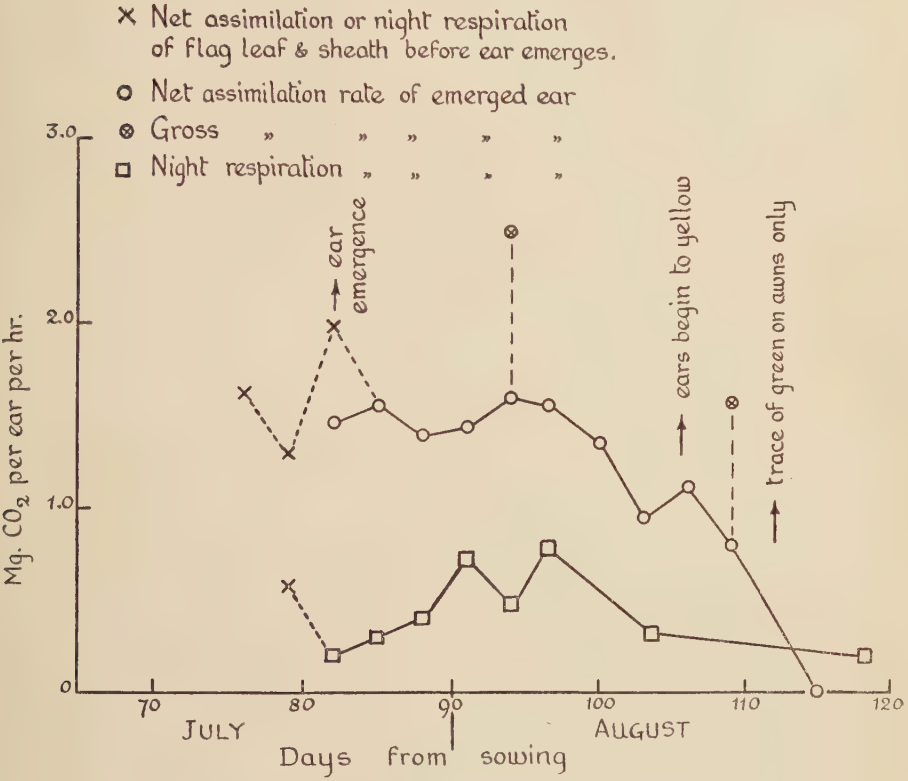

FIG. 3. Hourly rates per ear of gross assimilation, net assimilation, and night respiration in barley ears, during the period of ear development.

per ear due to photosynthesis in the ear and a net gain of hexose of 343 mg. per ear when allowance is made for the loss of carbon dioxide in night respiration. The gross assimilation figure is equivalent to about 700 mg. of hexose. The progress of accumulation of the products of photosynthesis in the ear is shown in Fig. 4 as running totals of the carbon dioxide absorbed, calculated as hexose, together with the dry weights of grain, and of awns, rachis, &c., and the starch content of the grain, determined at the end of each 3-day period on the ears whose assimilatory capacity had already been measured. The mean of 15 values for the dry weight of the ear at full ripeness was 1·30±0·05 g., of the grain 1·09±0·04 g., and for the starch content of the grain 649±35 mg., leaving 351 mg. per ear for other products accumulated in the grain, chiefly protein, and polysaccharides other than starch. It will be seen from Fig. 4 that after ear emergence only the grain increased in dry weight, the other organs of the ear reaching a maximum dry weight as the ear emerges. When the final dry-weight values are compared with the assimilation data it is found that the gross assimilation figure (i.e. the sum of the CO_2 absorbed by the ear and the CO_2 lost in day-time respiration as measured on darkened ears) is equivalent to 57 per cent. of the whole dry weight of the ear; the amount of CO_2 absorbed, or net assimilation, to 32 per cent.; and the net gain of hexose (i.e. net assimilation less CO_2 lost by night respiration) to 26 per cent.

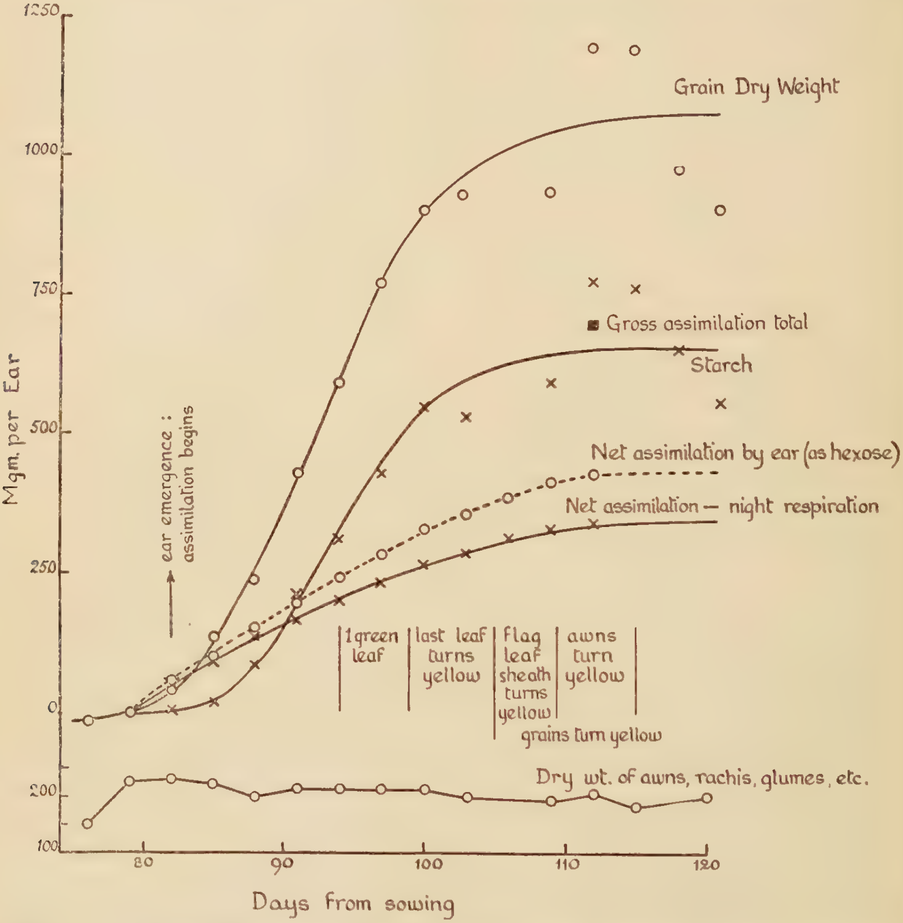

FIG. 4. Dry weights of grain, and of awns, rachis, &c., and the starch content of the grain of barley during ear growth, together with running totals of the carbon assimilated by the ear, expressed as hexose.

In terms of the amount of dry weight accumulated while the ear is assimilating, namely, 1,000 mg., the proportions are 70 per cent. for the gross figure, 42 per cent. for the net figure, and 34 per cent. for the net gain of hexose.

On the assumption that the substrate for respiration is provided by translocated sugar present in the ear at emergence together with sugar translocated during ear growth from other organs, then the gross assimilation by the ear could account for all the starch, while net assimilation is equivalent to 64 per cent. of the starch and the net gain in hexose to 52 per cent. On the other hand, the amount of non-starch products accumulated after ear emergence bears a close correspondence with the amount of hexose gained by assimilation in the ear, the figures being 351 mg. for accumulated non-starch material and 343 mg. for net hexose gain, so that the hypothesis that these compounds are in fact preferentially formed directly from the newly assimilated carbon must be considered as possible.

DISCUSSION

The data presented here afford confirmation of the results of earlier experiments with shaded ears, from which it was concluded that about 30 per cent. of the dry weight arose from autonomous assimilation, and show further that this contribution is made solely to the grain, there being no increase in the dry weight of other parts of the ear after its emergence. Since about 25 per cent. of the final dry weight of the ear is present at emergence, and 30 per cent. is contributed by its own assimilation, there remains about 45 per cent. to be supplied during ear growth by photosynthesis in other green organs. The assimilatory capacity of the flag leaf and its sheath together was found to be about equal to that of the emerged ear (see Fig. 3), and these organs remain green for about the same length of time as the ear. If, therefore, their assimilation rate also remains constant until the yellowing process begins, as does that of the ear, then the total contribution of the flag leaf and sheath will be of the same order as that of the ear, namely, 30 per cent., so that the bulk of the carbon not supplied at this time by the ear itself would be derived from this source. A number of earlier observations support this view; thus Archbold (1942) found that while removal of leaves only reduced the dry weight of the ear by amounts of 10 per cent. or less, additional removal of the flag-leaf sheath reduced the ear dry weight by 28 per cent. Watson and Norman (1939) record that shading either the ear or the rest of the shoot led to about equal reductions in the dry weight of the ear, indicating similar contributions by photosynthesis in the two cases, while Boonstra (1929) showed that in wheat only the top internode and peduncle, with their attached leaves, were effective in supplying material to the ear. Material for ear growth thus appears to be derived from assimilation in leaves and sheaths, before ear emergence, accounting for 25 per cent. of the final dry weight, by assimilation in the flag leaf and sheath, the top internode and peduncle after ear emergence accounting for 45 per cent. of the dry weight, the main contribution to this fraction coming from the flag-leaf sheath, and lastly by assimilation in the ear itself accounting for 30 per cent. of the dry weight.

Considering the accumulation of materials in the ear in more detail, inspection of Fig. 4 shows that as the ear emerges its net assimilation rate is for a short time sufficient to supply all the dry matter necessary for ear growth. As the rate of dry-weight accumulation increases, while ear assimilation rate remains constant, this phase is quickly terminated, and during the period of maximum growth rate large external supplies of carbohydrate are required. About 22 days after ear emergence, at which time growth rate begins to fall off, there is a period of 10 to 12 days in which the products of assimilation, though photosynthesis in the ear is declining, are again sufficient to supply the decreasing demands for growth. Rapid starch synthesis in the grain begins about 4 days after ear emergence, and the curve of starch increase has a sigmoid form similar to that of total dry weight, while the accumulation of non-starch materials (not separately shown in Fig. 4) proceeds at a nearly constant rate for about 23 days and then ceases. This fraction of the dry weight consists mainly of protein and carbohydrates other than starch (cellulose, &c.), and is collectively estimated as the difference between the total dry weight and the starch content. Both at the beginning and end of the growth period of the ear the net amount of autonomous assimilation is sufficient to supply the substrate for starch synthesis, but for a period of about ten days when starch is accumulating at the maximum rate the concomitant assimilation values fall short of the amount needed for starch synthesis.

It is of course impossible to deduce from the available data the precise fate of the carbon assimilated by the ear. As has been pointed out (p. 65), if only the final amounts are taken into account it would be possible, on the assumption that carbon dioxide respired during the day is at once reassimilated and converted to starch, for all the starch to arise from the photosynthetic activity of the ear. Moreover, in the barley ear sugar accumulates in the period before emergence and later declines to almost zero as the ear ripens. The amount of sugar accumulated is of the order of 100 mg. per ear and would thus be enough to supply the substrate for night respiration throughout the development of the grain, a process requiring about 80 mg. of hexose per ear. This hypothesis that carbon dioxide assimilated by the ear contributes to starch formation rather than to other products may possibly be supported by the observations of Harlan and Anthony (1920) that removal of the awns of barley diminished the dry weight of the ear by reducing the starch content of the grain, and of Watson and Norman (1939) and one of us (H. K. P. unpublished) that intake of nitrogen by the ear was little affected by shading this organ, so that reduction in dry weight when ears are prevented from assimilating falls upon the carbohydrate fraction. On the other hand, it is well known that detached leaves can form starch in the dark when given external supplies of sugar, so that it is not in all circumstances necessary, for starch synthesis, that assimilation shall be proceeding concurrently. As has been pointed out (p. 65), the close correspondence between the net gain in the ear by assimilation and the amount of non-starch products accumulated might suggest that it is indeed these materials which are preferentially formed from the autonomous assimilate.

It seems likely that organizing factors in the grain itself determine the nature of the products synthesized at any given time, irrespective of the nature and source of the carbohydrate supply. Thus in the early stage of ear development the products accumulated are mainly protein and cellulose type carbohydrates, and it may well be that all available carbohydrate supplies are directed to their synthesis, and there is in fact no discrimination between supplies arriving from organs other than the ear and those provided by the ear itself, as regards their use as substrates for formation of any of the required compounds. At present it can only be said with certainty that the ear is an important organ in contributing to the carbon supply for the grain, but the nature of the products to which it contributes remains a matter for speculation.

SUMMARY

A continuous record has been made of CO_2 assimilation by day and respiration by night of barley (var. Spratt Archer) ears attached to the plant, and kept under normal outdoor conditions. Assimilation proceeded for 33 days, during which 622 mg. of CO_2 per ear of average grain weight 1·09 g. were absorbed during 496 daylight hours and 117 mg. of CO_2 were emitted during 296 dark hours. Calculated in terms of hexose the net gain of carbon by autonomous assimilation was equivalent to 34 per cent. of the grain dry weight or 52 per cent. of the starch, a result in accordance with the estimates obtained in earlier experiments with shaded ears. On the assumption that sugars present in the ear at emergence or translocated to it during subsequent growth provide the substrate for respiration, the sum of CO_2 absorbed and the CO_2 formed by respiration in the day, as estimated on darkened ears, could account for 70 per cent. of the dry weight of the grain and all the starch.

About 30 per cent. of the grain consists of non-starch material, which might arise from the autonomous assimilate rather than starch. The fate of the CO_2 absorbed by the ear is discussed, and also the sources of the material required for its growth in addition to that supplied by its own assimilation.

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Phenol Oxidases in some Wood-rotting Fungi

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INTRODUCTION

RECENTLY Preston and McLennan (1948) described the decolorization of dyes by certain types of wood-rotting fungi. They showed that white wood-rotting fungi would decolorize a series of dyes, whereas brown wood-rotting fungi had no such effect. The decolorization was not due to a change in pH, and was not an effect of a reversible oxidation-reduction system. The dyes were irreversibly changed, and it was suggested that 'they are oxidised by the enzyme systems of the white rots'. These authors described an experiment which indicated that oxygen is necessary for the decolorization of dyes by white rots, and it seems probable that an oxidase system is involved, as has been suggested in the Bavendamm reaction (Davidson et al., 1938; Boswell, 1941). By this reaction white wood-rotting fungi have been distinguished from brown wood-rotting fungi by the use of certain test substances added to the media. The most common of these are tannic and gallic acids. The darkening of these acids by the white rots is stated to be due to an enzyme of the catechol oxidase type. Brown rots do not cause darkening of gallic or tannic acid media. However, the dye decolorization as described gives a more reliable differentiation between the types of wood rots.

Many wood-rotting fungi give various 'oxidase' reactions (Boswell, 1941; Garren, 1938; Bose and Sarkar, 1937; Venkatarayan, 1936). Bose and Sarkar (1937), on account of the positive guaiacum reaction, reported the presence of laccase in wood-rotting Polypores. La Fuze (1937) showed that *Polystictus versicolor* (L.) Fr. acts on guaiacum, resorcinol, and tannin. *Polyporus betulinus* Fr. and *Fomes pinicola* Fr. had no action on guaiacum, but *Fomes pinicola* acted slightly on tyrosine. These and other similar tests indicate the presence of phenol oxidases.

The object of this work was to try to identify the agent responsible for the decolorization of the dyes. Only low concentrations of dye are decolorized, and the rate is always very slow. Preliminary experiments with some of the white rots indicated that the dye decolorization might be related to the presence of phenol oxidases. Extracts were prepared and tested on a number of substances, including neutral red and gentian violet, which were selected by Preston and McLennan (1948) as representatives of the dyes which white rots decolorize.

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EXPERIMENTAL AND RESULTS

Species of both types of wood-rotting fungi were selected. These were grown on 2·5 per cent. liquid malt medium in 250-ml. conical flasks for periods of 3–8 weeks at 24° C.

The action of a water extract of the mycelium of several different species on the various substrates is shown in Table I.

TABLE I

Oxidizing Enzymes in Wood-rotting Fungi

	White rots.							Brown rots.			
	Polystictus versicolor.	*Ganoderma applanatum.*	*Fomes robustus.*	*Fomes rudis.*	*Stereum hirsutum.*	*Poria sp. 2.*	*Polyporus gilvus.*	*Polyporus anthracophilus.*	*Polyporus Eucalyptorum.*	*Coniophora cerebella.*	*Trametes lilacino-gilva.*
Guaiacum	+	+	+	+	+	+	+	+(−)	+(−)	−	−
Guaiacum +trace catechol	..	..	..	+	+	..	+	+	+	−	+(−)
Tyrosine	−	−	−	+(−)	+(−)	−	+	+	+	−	−
p-Phenylene diamine	+	+	+	+	+	+	+	+	+(−)	−	−
Hydroquinone	+	+	+	+(−)	+	+	−	+(−)	−	−	−
Pyrogallol	+	+	+	+	+	+	+	+	+	∧	−
Phenol	+	+	+	+	+	+	−	+	−	∧	−
Resorcinol	+	+	+	+	+	+	−	+(−)	−	∧	−
p-Cresol	− (milky)	− (milky)	−	+	+(−)	−	+	+	+	−	−
m-Cresol	− (milky)	− (milky)	− (milky)	−	−	− (milky)	−	+(−)	−	∧	−
o-Cresol	+	+	+	+	+	+	+	−	−	∧	−
Potassium ferrocyanide	+	+	+	+	∧	+	−	−	∧	∧	∧
Peroxidase	+	+	+	+	+	+	+	−	−	−	−
Catalase	−	+	−	−	−	−	−	+	−	−	−

+ indicates positive action
− indicates no action
+(−) indicates feeble action
∧ omitted

Where the action on guaiacum + trace of catechol was equal to the action on guaiacum alone no sign is given. A plus sign in this case indicates increased action.

Two brown rots (*Coniophora cerebella* Pers. and *Trametes lilacino-gilva* Berk.) showed no reaction on the substrates tested. Two other species (*Polyporus anthracophilus* Cooke and *Polyporus Eucalyptorum* Fr.) contain an enzyme which acts on a number of substrates including tyrosine. These reactions are similar to those described for the phenol oxidase 'tyrosinase' obtained from other sources (Nelson and Dawson, 1944). None of the brown rots gave a peroxidase reaction. Some white rots (*Polystictus versicolor* (L.) Fr., *Ganoderma applanatum* (Pers.) Pat., *Fomes robustus* Karst., and *Poria* sp. 2) contain a phenol oxidase which shows no action on tyrosine, but acts directly on guaiacum. Three species (*Fomes rudis* Berk., *Stereum hirsutum* (Willd.) Fr., and *Polyporus gilvus* Schw.) gave reactions which indicate the presence of more than one phenol oxidase.

Extracts of the white rots in most cases acted on the dyes to some extent, whereas extracts of the brown rots had no effect.

Examination of Table I suggests some possibilities regarding the enzyme concerned in the decolorization of the dyes. It seems obvious that the white rots contain a phenol oxidase differing from that which occurs in certain brown rots. The white rots contain also a strong peroxidase. Therefore, certain species of white rots were selected for further investigation. As *Polystictus versicolor* grew comparatively rapidly and was one of the most active species in decolorizing the dyes, it was most commonly used in the tests described. Some preparations were obtained also from *Ganoderma applanatum* and compared with those from *Polystictus versicolor*.

Preparation of phenol oxidase. Two methods were applied for the concentration and purification of the enzyme.

1. The mycelium was ground and extracted several times with water and the medium added to the extract. Acetone was added to a concentration of 50 per cent., the precipitate centrifuged off, and the remaining liquid increased to 70 per cent. acetone concentration. The precipitate from the 70 per cent. acetone was collected and dried. This method is similar to that used by Kubowitz (1937) in the first stages of his preparation of phenol oxidase. This product contained a phenol oxidase and gave a weak reaction for peroxidase. It acted on the dyes.

2. The majority of the enzyme preparations were made by the method of Dalton and Nelson (1939) without repeating the purifications. Ammonium sulphate was added to the aqueous extract until the solution was 0·6 saturated, the precipitate centrifuged off, suspended in water, and dialysed overnight. The solution was reprecipitated with the same concentration of ammonium sulphate, and the precipitate centrifuged, suspended in water, and dialysed against running water till free from ammonium sulphate. The phenol oxidase was adsorbed on to acid-washed kaolin at pH 5, with subsequent elution in phosphate buffer at pH 8·4. The eluate was dialysed against running water and finally against glass distilled water till almost free of phosphate.

The final preparations, unlike the original extracts, gave no peroxidase reaction. Though mostly adsorbed by the kaolin, peroxidase was not eluted by the phosphate. The final preparations acted on the dyes. This indicates that peroxidase was not a factor in the decolorization and the following observations support this conclusion:

1. Analyses of two preparations acting on the dyes gave the following results:

   Preparation from *Polystictus versicolor*: Cu, 0·15 μg./ml.; Fe, nil.
   ,, ,, *Ganoderma applanatum*: Cu, 0·5 μg./ml.; Fe, nil.

2. Peroxidase was prepared from horse-radish by the method of Keilin and Mann (1937). Solutions of this preparation tested over a pH range 4–6 caused no change in the dyes.

3. Some preparations of the white rots which gave a strong peroxidase reaction showed very little action on the dyes (e.g. *Polyporus gilvus*).

PHENOL OXIDASE

The preparations after elution and dialysis acted on the substrates given in Table I, and, therefore, contained a phenol oxidase. As indicated above, copper was present in these solutions.

1. *Decolorization of the dyes*

In order to investigate the relationship of the phenol oxidase to the dye destruction, concentrations of 0·001 per cent. of buffered dye were added to an equal volume of the enzyme preparation, and boiled controls prepared for comparison. Merthiosal (total concentration of 1 in 60,000) was added for prevention of bacterial growth. The tubes were incubated at 40° C.

The decolorization of the dyes by most of the extracts was very slow and often not complete. However, the change was quite definite when compared with the boiled controls which showed no apparent change over a period of several days. Solutions buffered at pH 4, 5, 6, and 7 all showed some action, pH 6 approximately being the optimum. Tests on the dyes were carried out at pH 6–6·1. The dyes changed very gradually over a period of several days, neutral red fading more rapidly than gentian violet.

The action of the enzyme on the dyes was tested in evacuated Thunberg tubes and compared with the action in air and with boiled controls. The dyes in the evacuated tubes remained the same colour as those of the boiled controls, while those in air changed gradually. The presence of oxygen is, therefore, required for the reaction. As mentioned previously, Preston and McLennan (1948) showed this with white rots grown on agar slopes.

The composition of the buffer affected the type of dye-change by the enzyme and concentrated buffer caused a slowing of the reaction. In general, phosphate buffer M/15 diluted 4 times was added. More concentrated buffer not only slowed the reaction but caused the gentian violet to develop a bright pink colour in the first stages of the change.

The action of the enzyme on catechol varied also with the type of buffer, as shown below. The uptake of oxygen of two preparations was measured, using equal amounts of two different buffers, pH 6·1.

	c.mm. O_2/ml./30 min.	
Preparation.	Phosphate buffer M/15.	Phosphate-citrate buffer (Sörensen).
Polystictus versicolor	27·3	17·5
Ganoderma applanatum (before purification by adsorption)	102	68·6

Similar effects of different buffers on other phenol oxidases have been reported (Boswell and Whiting, 1938).

The rate of paling of the dyes was related to the amount of enzyme preparation added. A high concentration of enzyme was always necessary for complete decolorization of the dyes. Small amounts failed to destroy completely the dyes even over periods of 2 weeks. Enzyme solutions remained active to some extent throughout this period, as shown by the addition of other substrates at the end of the test.

Concentration of the enzyme preparations in front of a fan at room temperature had a varying effect. In certain cases some activity was lost. Other preparations after concentration by this means showed a more rapid action on the dyes.

Table II

	Test solution.	Original preparation.	Preparation concentrated 5 times.
1.	1 ml. enzyme 1 ml. 0·001% neutral red	Decolorized 4 days	Decolorized 20 hours
2.	1 ml. enzyme 0·5 ml. 0·001% gentian violet	Changed, but not decolorized in 4 days	Almost decolorized in 4 days
	0·5 ml. enzyme added to 2	Colour still present 12 days later	Colour disappeared 2 days later

Attempts were made to detect uptake of oxygen during the dye-change. The action was so slow that results were considered unsatisfactory.

A series of estimations of uptake of oxygen were carried out on different preparations using catechol as substrate. At the same time the decolorization of the dyes by these preparations was followed qualitatively by comparison with boiled controls.

The reaction mixture for oxygen uptake consisted of buffer, 1 ml.; water, 1 ml.; enzyme, 1 ml.; catechol, 0·5 ml. of 2 per cent. solution added from the side arm. The experiments were carried out at 40° C.

Table III

	Preparation.	c.mm.O_2/ml./30 min.	Dye decolorization.
Compared together	*Polystictus versicolor* (1)	18·7	Dyes attacked slowly. Colour still definite in 6 days
	Polystictus versicolor (2)	31	Dyes attacked more rapidly. Colour much paler than in (1) in 6 days
Compared together	*Ganoderma applanatum* (1)	34·6	Dyes slowly attacked. Colour still present in 9 days in neutral red and gentian violet
	Polystictus versicolor (3)	70	Neutral red paled in 1 day, very slight colour after 4 days. Gentian violet paled, but colour present less degree than above in 9 days
	Ganoderma applanatum (2) (before adsorption on to kaolin)	102	Neutral red—change rapid, complete in 4 days. Gentian violet—more rapid change. Colour distinctly paler than above in 9 days

The changes produced in the dyes, whether complete or partial, appeared to be irreversible. Attempts to restore the colour by treatment with various reagents all failed.

A decolorization of dyes, similar to the enzymic effect, can be produced by hydrogen peroxide. Tubes containing 1 ml. of 0·001 per cent. neutral red or gentian violet were decolorized slowly when hydrogen peroxide was added to a concentration of 0·08 per cent. The dyes were attacked over a range of pH from 3 to 6, pH 6 being the optimum.

Some oxidation systems produce hydrogen peroxide and the possibility of its formation as a factor in the dye decolorization was investigated. Catalase, prepared by the method of Sumner and Dounce (as described by Sumner and Somers, 1947), was added to solutions of the dye and enzyme, the amount being sufficient to destroy comparatively large quantities of hydrogen peroxide. The presence of the catalase had no effect on the rate of decolorization of the dyes. The catalase was present at the end of the experiment, as indicated by the addition of hydrogen peroxide.

The slow rate of reaction on the dyes suggests that the products may act as inhibitors of the enzyme. Solutions of the dyes and enzyme were incubated as described, and, after 15 hours, when there was noticeable change in the colour of the dye, these solutions were divided into two equal volumes. One volume was dialysed for 24 hours, in order to remove soluble degradation products. A further amount of dye was added to both volumes and the tubes incubated. The rate of decolorization of the second volume of dye was equal in both cases. In neither the case of neutral red nor of gentian violet was an inhibitory action of breakdown products demonstrated by this means.

2. *Further properties of the phenol oxidase*

Effect of carbon monoxide. Enzyme preparations were treated with carbon monoxide, both in the light and in the dark. The action on the phenolic substrates and the dyes was not affected.

Effect of cyanide. Potassium cyanide M/1000 at pH 6 was added to the enzyme preparation. Decolorization of the dyes took place more slowly in the presence of cyanide. The action on other substrates, including catechol, was also slower in the presence of cyanide.

Effect of metallic ions. Salts of copper, iron, zinc, manganese, and magnesium had no effect on the dye decolorization by the enzyme. Under certain conditions gentian violet was decolorized by copper salts alone, but this change was reversible and the colour reappeared on standing. In the presence of the enzyme copper salts showed no such effect. The rate and amount of decolorization by the enzyme was not increased by the copper salts.

Effect of organic acids. Solutions of malic, citric, and fumaric acids at pH 6 showed no effect on the dye decolorization by the enzyme preparations.

Effect of catechol. Catalytic amounts of catechol, when added to the enzyme solutions, caused no increase in the rate of dye destruction.

Action on amino-acids. Certain phenol oxidases, in the presence of catalytic amounts of catechol, will convert amino-acids and amines into highly coloured compounds. Preparations from *Polystictus versicolor* and *Ganoderma applanatum* gave a red colour with glycine or di-methylamine, in the presence of catechol.

Preparations from these white rots acted directly on adrenalin, forming a red-coloured compound. However, the reaction was given more strongly by preparations from *Polyporus anthracophilus*, a brown rot.

Oxidation of tannic and gallic acids. Tannic and gallic acids (Bavendamm) buffered at pH 6 were added to the enzyme preparation and incubated at 40° C. The darkening of these acids started almost immediately and was distinct in an hour. The colour became intense overnight.

Peroxidase from horse-radish acted on these substrates very slightly and very slowly. The enzyme of *Polyporus anthracophilus* slowly attacked gallic and tannic acids.

Laccase from *Rhus succedanea.* Crude preparations of laccase from *Rhus succedanea* were obtained by treating the latex with acetone, centrifuging, and drying the precipitate. Solutions of these preparations acted on the various substrates described for laccase (Yakushigi, 1940; Gregg and Miller, 1940; Sumner and Somers, 1947). Some preparations acted very slightly on resorcinol and *o*-cresol, and others showed no reaction on these substrates. Gallic and tannic acids were oxidized by this enzyme. However, there was no action on the dyes even in high enzyme concentrations.

Phenol oxidase of brown rots. Four brown rots were examined for their phenol oxidase content. Of these, *Polyporus anthracophilus* and *Polyporus Eucalyptorum* acted on a number of phenolic substrates, including tyrosine (Table I). *Coniophora cerebella* and *Trametes lilacino-gilva* showed negligible action on the substrates tested.

Extracts of *Polyporus anthracophilus* were prepared as described for the white rots. These acted on the phenolic substrates as shown, but did not attack the dyes. As indicated already, gallic and tannic acids were slowly oxidized.

Discussion

Consideration of the above results indicates that the destruction of the dyes by the white rots is associated with the presence of a specific phenol oxidase. The preparations analysed contained copper but no iron. The slowing of the action on the dyes by cyanide is in accordance with the inhibitory effect of cyanide on phenol oxidases from other sources. Keilin (1929), Keilin and Mann (1938), and others have shown that the polyphenol oxidases from potato and mushroom are inhibited by potassium cyanide. They showed also (1939) that the laccase of *Rhus succedanea* was inhibited by cyanide, and Gregg and Miller (1940) obtained a similar result with laccase from the wild mushroom.

The increase of dye destruction with increased rate of catechol oxidation also indicates a close relationship with a phenol oxidase. The action on the dyes is very slow compared to the oxidation of the phenolic substrates and this might be due to the formation of some inhibitory substance. However, dialysis after some decolorization had occurred had no effect in hastening the decolorization of further amounts of dye. If inhibitory substances are formed they are not removed by dialysis. There is no evidence for an enzyme system involving other substances such as catechol. Catalytic amounts of catechol did not increase the rate of dye destruction, and, moreover, all soluble constituents would be removed from the enzyme solution during the long dialyses. The dyes and potassium ferrocyanide differ entirely in constitution from each other and from the phenolic substrates, and the enzyme, therefore, may have various modes of attack. Further examination of the dye-decomposition products is necessary to show what is involved in the decolorization by the enzyme.

The white rots examined all contain a phenol oxidase. Some, according to their actions, may contain a mixture of phenol oxidases. There is a great variation in the rate of dye destruction in the several species tried, and this is possibly due to the amount of enzyme produced. *Polyporus gilvus*, which showed a very weak action on the phenolic substrates, had a negligible effect on the dyes, whereas *Polystictus versicolor*, which acted strongly on the phenolic substrates, had a marked effect on the dyes.

The rate of dye destruction on agar slopes is very slow compared with the oxidation of catechol, indicating also that high concentrations of enzyme are necessary for the decolorization. The enzyme preparations were weak in their action compared to the mycelium on agar slopes. This is probably due to the fact that complete extraction of the enzyme was never achieved, and a great deal of activity lost in the process of purification.

The phenol oxidase which decolorizes the dyes acts strongly on gallic and tannic acids and is, therefore, also responsible for Bavendamm's test which is given so readily by the white rots.

The phenol oxidase of the white rots is more closely related to 'laccase' than to 'tyrosinase'. This is indicated by its substrate action and by the fact that it is unaffected by carbon monoxide. Keilin (1929) and Keilin and Mann (1938) showed that polyphenol oxidases, often termed 'tyrosinase', are inhibited by carbon monoxide whereas the laccase of *Rhus succedanea* (Keilin and Mann, 1939) is unaffected. Laccase from the wild mushroom (Gregg and Miller, 1940) is reported to oxidize potassium ferrocyanide and this action was given by the white rots. Laccase from *Rhus succedanea* also acted on gallic and tannic acids. However, unlike the phenol oxidase in the white rots, it showed practically no action on resorcinol or on *o*-cresol and did not attack the dyes. Yakushigi (1940) prepared laccase from *Lacterius piperatus* and showed that it acted on resorcinol, but not on *o*-cresol. It is evident that these related enzymes from different sources show a considerable variation in their substrate specificity.

Some brown rots, according to their reactions, contain an enzyme more closely related to 'tyrosinase', but brown rots, as shown by Preston and

McLennan (1948), do not decolorize the dyes. *Polyporus anthracophilus* gave very strong tyrosinase reactions, and in concentrated preparations showed no action on the dyes. In some other brown rots, e.g. *Coniophora cerebella*, a potent rot, phenol oxidase, if present, is not in an active form.

The presence of phenol oxidases in wood rots has been associated with a variation in the type of rot produced (Campbell, 1930; Boswell, 1941; Cartwright and Findlay, 1944), and, in the small field examined, there is distinct difference in the distribution of these enzymes between the white and the brown rots. Boswell and Whiting (1938) and Baker and Nelson (1943) have shown that the phenol oxidase system is concerned in the respiration of the potato tuber. This function was further investigated by Boswell (1945) and by Robinson and Nelson (1944), and Walter and Nelson (1945) have shown that tyrosinase has the role of a terminal oxidase in the sweet potato. A further study of these phenol oxidases in the wood rots might indicate whether or not they play such a part in these fungi.

SUMMARY

1. The decolorization of dyes by white wood-rotting fungi is attributed to a specific phenol oxidase. Brown rots, on the other hand, cause no decolorization and do not contain this enzyme in significant amounts.

2. Some properties of the phenol oxidase of white rots have been studied and its action is shown to be similar in certain respects to that of laccase, though the substrate specificity differs.

3. Some brown rots contain a phenol oxidase which, according to its reactions, resembles tyrosinase of mushroom and potato. Other brown rots examined showed a negligible phenol oxidase reaction.

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Changes in the Drought Resistance of Wheat Seedlings during Germination

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With four Figures in the Text

IT is generally agreed that dormant embryos are extremely resistant whereas seedlings are relatively susceptible to desiccation; the stages of germination therefore provide an organized plant system which during development displays rapidly decreasing drought resistance. A study of the reaction of seedlings to desiccation during these different stages should thus clarify some aspects of drought resistance. It was the aim of the present investigations to make a preliminary survey of such behaviour; primarily, to ascertain the rate of change in drought resistance of dormant wheat embryos from germination onwards, and to correlate such changes with stages in seedling development.

METHODS

Wheat seedlings were germinated and grown on stainless-steel wire gauze supports. Each support consisted of a square of 12-mesh gauze, suspended from glass stands by stainless steel wire, and held 12 grains in meshes which had been suitably enlarged. The height of each support was adjusted so that the basal one-third of the grain was immersed in a nutrient solution contained in glass dishes. At appropriate stages of germination a number of the seedlings were removed for drying. The number of seedlings was reduced to 8 of uniform size per gauze. The gauzes were then randomized for treatments and suspended over concentrated sulphuric acid in desiccators. At suitable intervals the gauzes were removed from the desiccators, half of the seedlings taken out at random for determination of moisture content, and the remainder immersed in a solution for recovery.

Before determining moisture contents the embryos were separated from the endosperms with the aid of a dissecting microscope. In expt. 1, moisture contents were obtained after drying at 95° C. for 24 hours. In expt. 2, the plant material was placed in small weighing-bottles on an electrically heated wire mat maintained at 50° C. All weighings were made on a Bunge micro-balance.

The remaining seedlings were kept fully immersed in solution for 24 hours in order to regain turgidity; the root and shoot lengths were then measured and the seedlings grown for a further 8 days. Measurements of shoot and root

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[1] This work was carried out while the author held a Farrer Memorial Scholarship. It formed part of a thesis submitted to the University of London for the Ph.D. degree.

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length were made at intervals of 2 days and the amount of growth made over 8 days taken as a measure of resistance to drying. The technique of estimating drought resistance by measuring the resumption of growth after a period of drought was first used by Ashby and May (1941).

RESULTS

Experiment 1

Two Australian varieties—Bencubbin and Charter, which are reputed respectively to be drought-resistant and drought-susceptible—were subjected to different degrees of desiccation at stages of growth corresponding to 3, 5, 9, and 17 days from the beginning of soaking. The degrees of drying were those which occurred when the seedlings were suspended in desiccators over concentrated sulphuric acid at a temperature of 19·3° C. for 3, 9, 27, and 81 hours.

Germination, growth, and recovery from drying were all carried out in a 0·002 M $CaCl_2$ solution. The 3-, 5-, and 9-day plants were kept throughout in diffuse daylight of a laboratory at a temperature of 14–15° C.; the 17-day plants were grown under these conditions for 12 days and were then removed to a greenhouse exposed to full winter daylight and with a temperature of 3–12° C. The plants remained here for the last 5 days of pre-drying growth and for the growth period following recovery. As no significant differences could be detected between the two varieties, means only for varieties are presented in the results.

TABLE I

Size of Plants at Time of Desiccation

Age (days).	Shoot length (mm.).	Total root length (mm.).	No. roots per plant.	Dry wt. embryo (mg.).
3	3	9	3	1·5
5	11	41	4	4·2
9	38	75	6	15·0
17	94	95	7	27·4

The sizes of seedlings at the times of desiccation are shown in Table I. Drying for the same period of time resulted in different relative amounts of water loss from the seedlings of different ages (Table II), but an extremely wide range of desiccation was obtained in all the sets.

The time of onset of wilting could not be observed accurately in the short coleoptiles of the 3- and 5-day-old plants. In the 9- and 17-day plants incipient wilting was observed after about 3 or 4 hours of drying. Most of the stomata were then closed, although a few remained permanently open. Stomata of 9- and 17-day plants dried for 81 hours did not reopen even after 2 days' immersion following drying. The shoots in these treatments did not regain turgidity during this period, although when kept for several days longer with the roots only in water, elongation of the second leaf, which was in the primordial stage at the time of desiccation, was observed. The second leaf initial did not appear to be damaged by this severe desiccation and

TABLE II

Shoot and Root Growth made by Seedlings of Different Ages after Recovery from Drying to the Moisture Contents shown

Age (days).	Hours of drying.	Water content after drying (% dry weight).	Percentage water lost on drying.	Growth made on recovery (mm. in 8 days).	
				Shoot.	Root.
3	0	450	0	63	77
	3	180	60	51	48
	9	36	92	40	28
	27	10	98	13	4
	81	4	99	2	0
5	0	530	0	68	55
	3	333	35	64	39
	9	168	66	45	23
	27	47	91	35	7
	81	5	99	4	0
9	0	670	0	57	44
	3	452	32	51	7
	9	222	67	42	7
	27	73	88	30	5
	81	31	95	0	0
17	0	675	0	59	21
	3	413	39	40	8
	9	228	66	31	7
	27	77	88	19	0
	81	15	98	0	0

sufficient water was absorbed through the roots or mesocotyl to allow its elongation to continue.

The plumules of the 3- and 5-day plants dried for 81 hours regained turgidity and slight elongation continued. Plumules so treated were very corrugated as if cells in localized areas were permanently injured, while in others they had regained their previous turgid state and continued to elongate. The cells in some areas were flattened and buckled, the normal shape and volume not being regained. A similar corrugated appearance was noticed in a few areas on the leaves of the 9- and 17-day plants dried for 27 hours.

Both root and leaf cells were folded and wrinkled after drying for about 9 hours in the 9- and 17-day plants, and after about 30 hours in the 3- and 5-day plants. Coagulation of the protoplasm did not appear until the plants had been dried for a longer period. Folding of the cell-walls seemed to be more severe in leaf tissue than in root-cells. Root-hairs of plants of all treatments no longer showed folding of cell-walls after immersion for a few minutes in water. The roots in all treatments, even those dried for 81 hours, became turgid within a few minutes of replacement in water, whereas the leaves and coleoptiles of the 9- and 17-day plants dried for 81 hours did not become turgid even after 2 days in water. However, this apparent resumption of turgidity by the roots was not followed by further growth.

The shoots of seedlings of all ages dried for 3 and 9 hours regained their original moisture contents within 24 hours of immersion in 0·002 M $CaCl_2$ solution, but this was not true of seedlings dried for longer periods. However, recovery in all treatments was taken as being completed at 24 hours after replacing in the solution and this time was used as the datum line for purposes of growth measurements. Shoot-growth of undroughted plants in this experiment was approximately linear, so arithmetic differences of the lengths at the end and beginning of the growth period following recovery were taken as measures of growth.

The roots of 3- and 5-day old plants dried for a period of 3 hours grew at a slower rate than roots of undroughted plants, and those dried for 9 hours grew even more slowly (Table II). There was a lag period of 2 days in the latter before growth was resumed. Roots of plants droughted for 27 hours did not recommence growth until about 6 days after recovery. Measurements of the separate roots were not made in this experiment, so it is impossible to state if roots existing at the time of drying were permanently injured (cf. expt. 2).

Experiment 2

Seedlings of one variety (Bencubbin) at stages of 1, 3, 5, 7, and 9 days from the commencement of soaking were dried in desiccators containing concentrated sulphuric acid for periods of 0, 2, 5, 10, and 30 hours. The entire experiment was carried out in the diffuse light of a laboratory at a temperature ranging from 8° to 12° C. Seedlings were germinated and grown throughout with daily changes of a culture solution containing (in p.p.m.) 252 $Na_2HPO_4 . 12\ H_2O$, 100 $CaCl_2$, 185 K_2SO_4, 910 $NaNO_3$, 125 $MgSO_4 . 5\ H_2O$, 279 $Fe_2\ (C_4H_4O_6)_3 . H_2O$, and adjusted to pH 5·5.

The sizes of seedlings at the times of desiccation are presented in Table III and are shown diagrammatically in Fig. 2. Seedlings of 1 day corresponded to the time of rupture of the pericarp.

TABLE III

Size of Embryos at Time of Desiccation

Age (days).	Shoot length (mm.).	Total root length (mm.).	No. roots per plant.	Dry wt. (mg.).
1	2	—	—	0·60
3	3	5	3	0·90
5	5	19	3	1·52
7	8	37	3	2·87
9	21	88	5	4·88

The relative rates of moisture loss during drying were similar for the 1-, 3-, and 5-day plants resulting in different absolute values, but there was a slower rate of loss from the 7- and 9-day plants (Table IV). Roots lost moisture much more rapidly than shoots, as shown by the data for 9-day treatments in which the moisture contents of roots and shoots were deter-

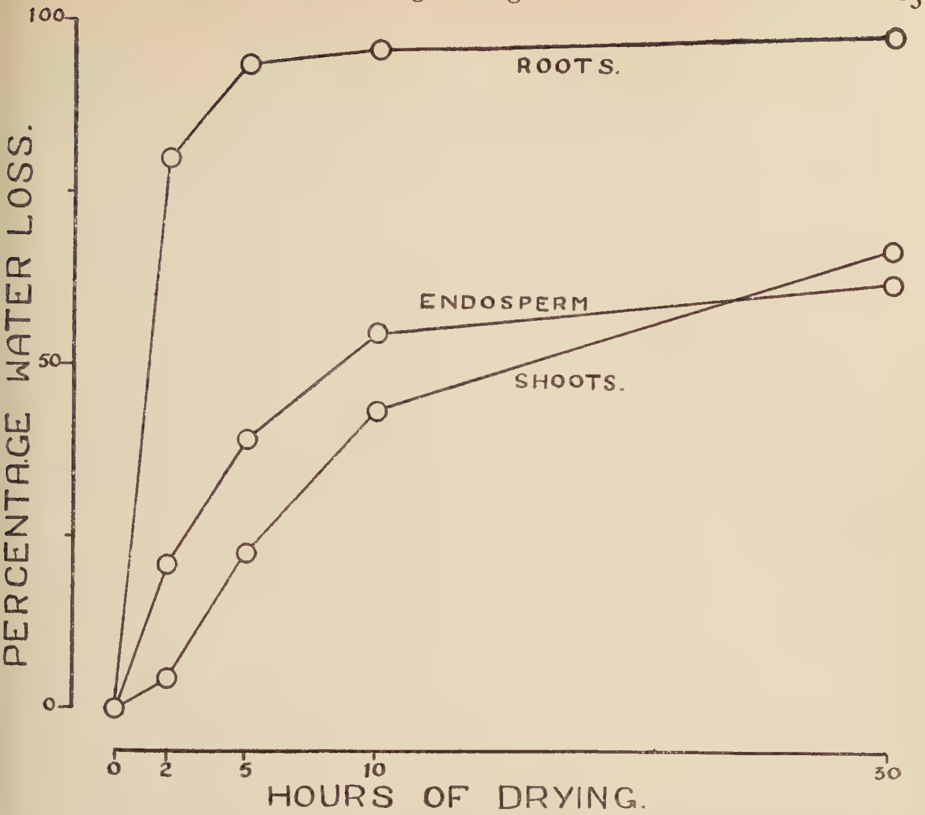

FIG. 1. Percentage loss of moisture by roots, shoots, and endosperm of 9-day-old plants after drying for different periods over concentrated sulphuric acid.

mined separately (Fig. 1). The much more rapid desiccation of the root than of the shoot may be attributed to the different surface/volume relationships of the two tissues and the absence of cuticle in the root. The data given in Table IV, therefore, do not give a correct picture of the moisture contents of the roots in the various treatments. Because of the low proportion of root to shoot, the values of moisture content of the whole embryos nevertheless correspond very closely to those of the shoots alone.

TABLE IV

Moisture Contents of Seedlings of Different Ages after Drying for Varying Periods (% dry wt.)

Age (days).	Hours of drying.				
	0.	2.	5.	10.	30.
1	294	163	53	17	8
3	531	270	120	41	8
5	637	256	108	74	13
7	756	407	245	157	57
9	784	549	372	266	149

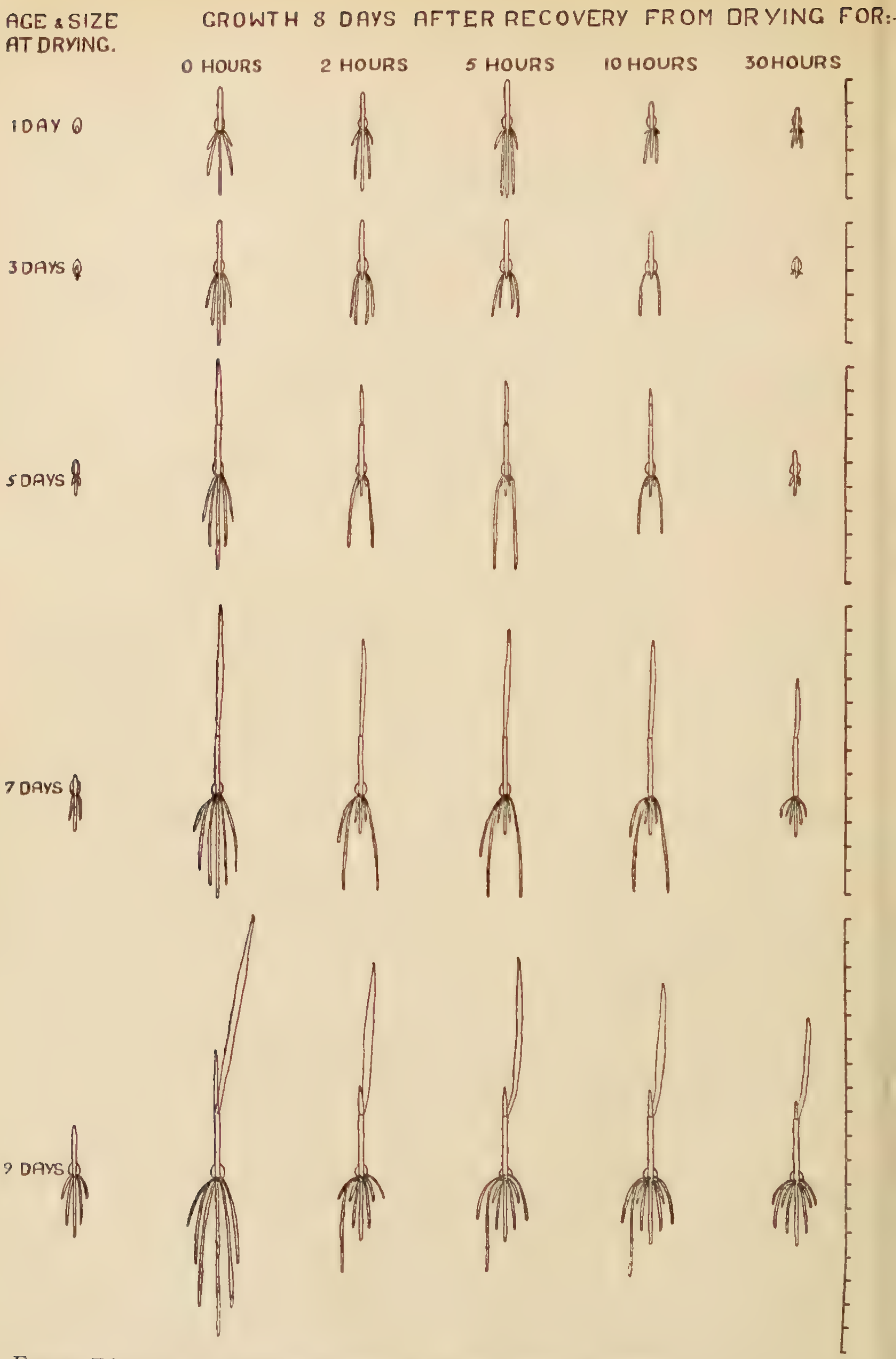

Fig. 2. Diagram showing mean lengths of different organs of seedlings (coleoptile, 1st and 2nd leaves, 1st root, 2nd and 3rd roots, and 4th, 5th, and 6th roots) at time of desiccation, and the lengths of these organs after 8 days' growth following drying for varying periods. (Each division on scale equals 1 cm.)

The amount of growth made in 8 days by the component organs of seedlings following recovery from drying was determined (Fig. 2). Roots of 2 mm. or more in length were completely killed by the least severe desiccating treatment, which probably represented a loss of moisture of about 80 per cent. Root initials, however, were not damaged even when the embryos had lost about 98 per cent. of their moisture. Roots 1–2 mm. in length (cf. 3-day series) were retarded in growth when the embryo had lost 77 per cent. of its moisture and were completely killed by a loss of 92 per cent. of the moisture. The effect of severe desiccation on the root initials was to lengthen the time before the young root appeared; once growth recommenced it continued at a rate comparable with that of undroughted plants.

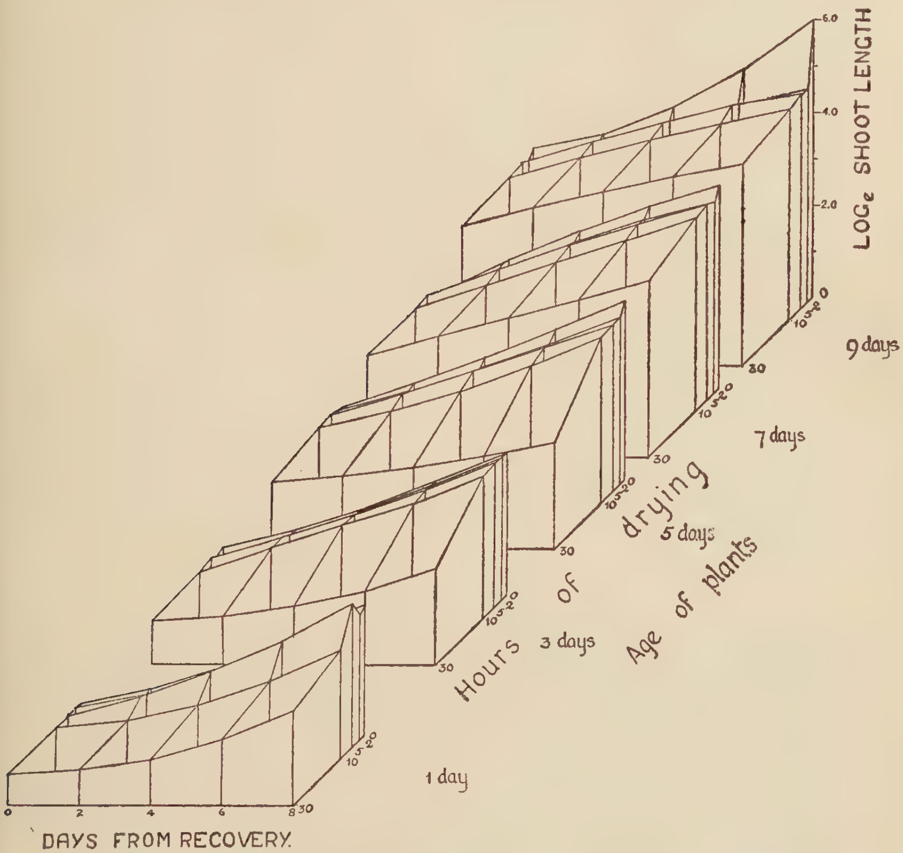

FIG. 3. Series of five diagrams of three-dimensional models showing logarithms of shoot-length of seedlings at 2-day intervals following recovery from drying for varying periods over concentrated sulphuric acid. Each model shows all treatments within one age-group; the values used in the diagrams were calculated from second-order equations fitted to the observed values for each treatment.

The data of lengths of shoot, after transformation to logarithms, were subjected to analysis of variance. The interactions with time from recovery were partitioned by the use of orthogonal functions (Cochran, 1939). The interaction of chief interest is that of 'age at drying × hours of drying × times of recovery after drying'; this showed significant differences in both the linear and quadratic terms. These differences are shown in Fig. 3, in which are presented the second-order regression equations of log. shoot length on time from recovery. The significant positive quadratic term indicated that elongation, particularly of undroughted plants, proceeded at a rate greater than exponential. Seedlings up to 5 mm. in length were not greatly affected by drying for 5 hours or less (i.e. with a loss of less than 80 per cent. moisture); more severe drying caused a lag in resumption of the initial growth rate, but this rate was resumed in the 1-day plants. In the older seedlings, however, the growth rate was retarded over the whole period and there was no indication of the former growth rate being resumed. The reduction in growth rate was shown even by the least-severely dried plants, but there were no significant differences between the 2-, 5-, and 10-hour treatments. Drying for 30 hours in the older seedlings (5-, 7-, and 9-days) resulted in further reduction in the growth rate.

A comparison of the relative growth rates with water-content indicated that the 1- to 7-day seedlings showed a marked reduction in growth with a loss of about 85 per cent. of the moisture. The growth of the 9-day seedlings was greatly retarded by a much smaller water loss. In general, the older the seedling the less water was lost before a reduction in growth rate was evident.

DISCUSSION

The early stages of growth of wheat seedlings showed three periods during which their behaviour after drying differed greatly. The first phase (A, Fig. 4) covered the time from the beginning of soaking to the stage when the coleoptile was about 3–4 mm. long. During this period there was a rapid absorption of water in the first hour, the water-content rising from *c.* 8 per cent. to *c.* 120 per cent. on a dry-weight basis. This probably represented imbibition by pre-existing cells of the embryo. The absorption rate, which had fallen after the first hour, gradually increased after about 4 hours and became very rapid up to the time of rupture of the pericarp. This high absorption rate continued until the moisture content was about 550 per cent. dry weight. This period probably included the time when most of the coleoptile cells were laid down (Avery and Burkholder, 1936), and during the whole of the period most of the plumule cells must have been in a meristematic state. The primary root appeared soon after the rupture of the pericarp and at the end of the period A was 3–4 mm. long. Severe drying at the end of period A killed the longer roots, but did not permanently injure the plumule. A loss of 85 per cent. or more of the moisture during this phase caused a lag in resumption of growth; but growth was gradually resumed and finally proceeded at a rate comparable with undroughted plants. This check in growth following severe desiccation suggests a dislocation in cellular organization, but the subsequent resumption of growth at the original rate indicates that it was only of a temporary nature. Wheat seedlings during this stage, therefore, may be regarded as being entirely drought-resistant.

The second phase of growth in relation to seedling behaviour after desiccation (B, Fig. 4) embraced the period of elongation of the coleoptile to the time of emergence of the first leaf. Cell-division probably continued during the early part of this period, but most of the elongation is likely to have resulted from extension of pre-existing cells in the basal region of the coleoptile (Avery and Burkholder, 1936). The rate of water absorption during this period was decreasing, but the water-content rose from about 550 to 800 per cent. dry weight. Slight drying resulted in death of emerged roots but did not injure the initials, which gradually grew out and expanded at a rate comparable with those of undroughted plants. Throughout this period there was a decrease in the growth rate of plants which had been subjected to equal degrees of desiccation. A loss of 60 per cent. of total water caused a slight reduction in the growth rate and this effect became more pronounced with increased desiccation. Loss of 98 per cent. of the total moisture, however, did not permanently injure the plant.

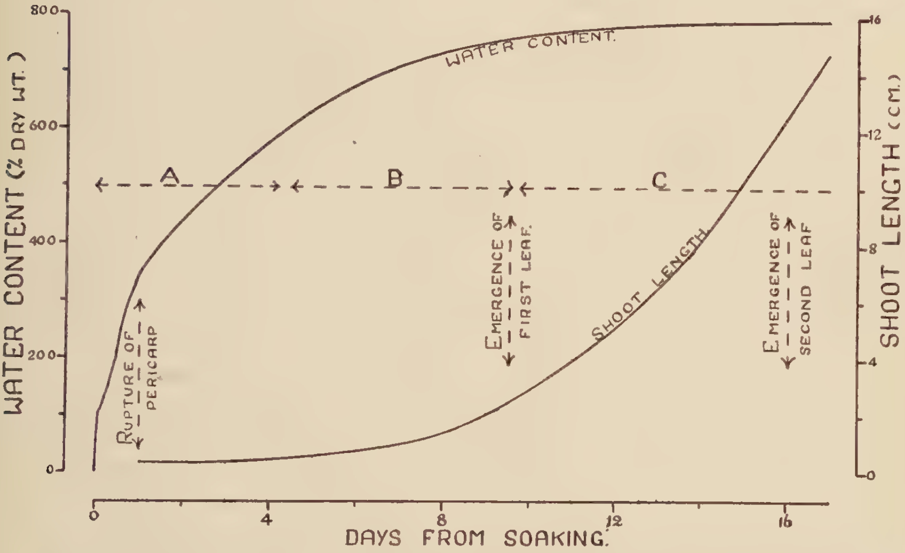

FIG. 4. Diagram showing the approximate duration of the three phases of drought resistance (A, B, and C) discussed in the text in relation to shoot-length and water-content. The values entered are calculated for 12° C.

Period C (Fig. 4) extended from the emergence of the first leaf to the end of the experiment. During this phase the moisture content remained fairly constant. Root reaction to drying resembled that in period B. The expanded tissue during this period was permanently injured by a loss of 92 to 98 per cent. of the total moisture and did not regain turgidity when immersed in water. Leaf primordia, however, were not killed and it is believed that under suitable conditions both root and leaf tissue would be regenerated. Loss of only 30 per cent. of the moisture retarded growth and the growth rate appeared to be permanently reduced by this degree of desiccation. In atmospheres of equal drying power, plants of this phase appeared to lose water more slowly than younger plants. This, which may be related to cuticle development, offsets, to some extent, their greater susceptibility to loss of moisture.

The evidence from these experiments suggests that meristematic tissue is completely resistant to desiccation; both root and leaf primordia can be dried to the condition of the unimbibed embryo without injury. The increasing susceptibility of seedlings with age may thus be related to the increasing proportion of vacuolated to meristematic cells.

The failure of vacuolated cells to recover from a substantial loss of water is probably due to death of the protoplast following mechanical injury. The experiments of Iljin (1927, 1933, 1935) suggest that death of plant-cells on drying results entirely from mechanical rupture following rapid changes in volume. Adhesion of the protoplast to the cell-wall probably plays an important part. The entirely different behaviour of the cell-walls of root and of shoot following severe drying suggests that these tissues would provide valuable material for an extension of these investigations on the causes of death of cells by drying. Root tissue readily resumes its original configuration following immersion after drying, while shoot-cells remain wrinkled and folded. This may indicate an inherent difference in the properties of the cellulose in the two tissues, or it may result from a less rigid attachment of the protoplast to the cell-wall in the root than in the shoot.

The wheat seedling retains its drought-resistant properties up to a stage marked by the rapid extension of the first leaf. Susceptibility to drought then increases markedly, but capacity to recover from severe drying is not altogether lost. So long as some root primordia remain in a meristematic condition, eventual recovery of the plant from drought is still possible, for the leaf primordia can resume growth if functional roots are present to effect the necessary water uptake. The persistence of roots in the primordial condition during the early stages of growth is no doubt very important in the drought resistance of the seedling stage of cereals and grasses, and the normal development of the seminal roots in succession may be regarded as a characteristic affording protection against temporary water shortage.

SUMMARY

Seedlings of two varieties, one reputedly resistant and the other susceptible to drought, were desiccated to varying degrees at different ages, and their growth response on recovery studied. No varietal difference was recorded; both were highly resistant to drying and showed three distinct age-periods of varying reaction.

All germination stages from the dormant embryo until the coleoptile reached a length of 3–4 mm. were completely resistant to drought, as shown by elongation of severely dried plants proceeding on recovery at a rate comparable with undroughted plants. Until the emergence of the first leaf, plants were not permanently injured by a loss of 98 per cent. of the total moisture, although elongating roots were killed. At later stages less water was required to be lost before expanded tissue was killed and the growth rate was permanently reduced by a small loss of water.

These phases of differential drought resistance appeared to be related to the proportion of meristematic to elongated cells, the former apparently being completely resistant. The persistence of root primordia during the early growth stages would thus appear to be an important factor in the drought resistance of Graminaceous seedlings.

Some aspects of the behaviour of tissues during desiccation and recovery are discussed.

This work was carried out under the direction of Professor F. G. Gregory, F.R.S., to whom the writer is greatly indebted for suggesting the subject for the research and for his stimulating interest and criticism during its prosecution. The investigations were made while the writer held a Farrer Memorial Scholarship; to the Farrer Memorial Trust his most grateful thanks are extended.

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Quantitative Evolution

XVI. Increase of Species-number in Diatoms

BY

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With four Figures in the Text

GENERAL CONSIDERATIONS

THERE now seems to be a reasonable amount of agreement concerning many of the principles of quantitative evolution, but a brief statement of the present position may be useful for those who have not followed the recent developments in detail.

Duration. One of the main phenomena upon which attention has been directed is the occurrence of long durations for many species of both plants and animals. This point has been summarized by Mayr (1942, p. 244) thus: 'Fossil evidence indicates that the "life span" (the time between origin and extinction) of some species is very long; for others, it seems short. Few tabulations exist as yet of probable "life spans"'. One extensive tabulation of known 'life spans' for species of diatoms (Small, 1945*b*) and another of known 'stratigraphical ranges' for important genera of foraminifera (Glaessner, 1945) have since been published, while Cushman (1940 and 1948) gave generic geological ranges for all foraminifera, and Cushman and Ozawa (1930) gave species-durations for all the Polymorphinidae, a family of foraminifera.

These and other data support Mayr's statements (1942, p. 223) that 'speciation in marine animals moves at a snail's pace'; 'The paleontology of marine animals indicates the same slow evolution as does the existence of so many bipolar species'; 'The speed of evolution should not be over-rated, even in terrestrial groups. Many of the insects of the mid-tertiary Baltic amber cannot be separated specifically from living species.'

De Wildeman (1947) does not agree with the idea of long durations for some species, but he accepts quite willingly the idea of some extinctions being due to innate causes, such as spontaneous sterility.

So far as generic durations are concerned, it has long been recognized that many living genera had their origins in early Tertiary or even in pre-Tertiary times (Darrah, 1939; Simpson, 1944, p. 21). So far as species are concerned, the recognition that many living species have continued for many millions of years specifically unchanged is a relatively recent phase which contrasts markedly with the still-prevalent idea that all species are in a state of genetical and morphological flux. The evidence is mainly palaeontological and factual,

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and includes the above-mentioned amber insects, also molluscs (see Davies, 1934), foraminifera, and in considerable detail the diatoms.

Glaessner (1945, p. 6) considers that micropalaeontology 'appears to be about to take a leading part in the exploration of the organic world of the past', and (op. cit., p. 26) quotes Ellison on the conodonts thus: 'He came to the conclusion that, *as in many other groups of fossils*, most of the genera and species are long-ranging but that certain types (platform types) are valuable zone markers.'

Utilitarian palaeontology has been so concentrated upon 'zone markers' that the significance of long-ranging species has been largely neglected in evolutionary theorizing, although the 'father of stratigraphy', William Smith, found that some of the fossils in any particular bed might be the same as some of those from the beds above or below, while others might be peculiar to one bed (Holmes, 1944, p. 99).

Long-lived species of angiosperms are now being recognized, and a fossil forest of Upper Miocene age at Joursac in France, complete with fossil fungi, mosses, conifers, and angiosperm woody plants, was described by Hèribaud and Marty (1903). This included living species of birch, alder, hornbeam, hazel, beech, oak, willow, poplar, ash, walnut, &c. Modern techniques for microscopic identifications of leaves have established specific identities for Miocene fossil specimens of living species (cf. Axelrod, 1948, p. 133).

The more recent outlook upon these long durations for angiosperm species is to be found in papers by Chaney, Babcock, Stebbins, &c., and the general trend is for present geographical distribution to be used as suitable evidence for pre-Pleistocene distributions of the same species. Stebbins (1947) quotes a Miocene species, *Fothergilla Gardeni*, as living now in America; *Metasequoia* and *Latimeria* are other outstanding examples of ancient types for conifers and fish respectively; but the large Miocene specific element in our modern flora is only beginning to be recognized. 'In fact, we can safely make the generalization that within the Arcto-Tertiary forest the evolution of herbaceous elements of the flora has been as slow as that of woody species' (Stebbins, 1947, p. 152).

Babcock (1947, Pt. I, p. 138) has a section on the probable status of the genus *Crepis* in Miocene, in which he gives an early Miocene existence for at least twenty-one living primitive rhizomatous species in various parts of Asia, and a late Miocene existence for seventeen more living species in the same part of the world. In fact, once the slowness of speciation is recognized in terms of millions of years, the presence of ancient lineages, as species of long duration, becomes a necessity dictated by limitations of available time for most large genera. The old genera need not necessarily be large, as can be seen in diatom generic histories (Small, 1945*b*), a phenomenon which is recognized for angiosperms by Stebbins (1947, p. 156) where he states that 'groups with preadapted gene combinations, in order to undergo rapid evolution, must also be able to evolve reproductive isolating mechanisms. Some xerophytic groups of coastal California, like the genera *Yucca*, *Heteromeles*, *Adenostoma*, and

Erysimum, have undoubtedly become more abundant in individuals in recent times, but nevertheless have not produced many species.'

Zeuner (1946*a*, p. 377) makes the same point thus: 'The one fact that emerges with certainty from these comparisons of faunas is, that, in marine faunas, a large number of species and subspecies are stable, since they have not noticeably modified their characters since the late Tertiary.' He then gives evidence concerning Javan Miocene deposits, which suggests that 30 million years (30 m.y.) is a maximum duration for any one species; but some geological ranges of diatom species extend beyond 70 m.y. from upper Cretaceous to Recent, and a few foraminiferal species have had even longer life spans, such as from the top of lower Cretaceous to Recent or about 100 m.y., which is also the maximum species-duration indicated in studies of quantitative evolution among diatoms.

Speciation. Long species-duration is commonly taken as proof of no evolution or very slow evolution, but duration of a parental species is no measure of evolution when parental species are recognized as giving rise to other species by changes which affect only one or a few individuals while the parent species continues unchanged.

This results in monopodial evolution, or ancestral species coexisting with their derived species even into Recent times, as found for diatoms (Small, 1945–8) and as pictured for the genus *Crepis* (Babcock, 1947). The mechanism of this monopodial evolution is summarized by Babcock (op. cit., p. 145). The primary genetic processes causing evolution are there given as (1) gene mutations producing morphological and physiological differentiations and sterility barriers; and (2) some structural chromosome changes which act in 'the genesis of intraspecific sterility leading to discontinuity and hence to speciation'.

A combination of both these processes could occur in one or a few steps, and the second process is known to involve only one or a few generations; and since there is much evidence for the increase in numbers of species being very slow in time, the process of speciation must take place with long intervals of time between each rapid speciation. This rapidity of speciation as contrasted with very long intervals of specific stability has been discussed by Goldschmidt (1940), Simpson (1944), Just (1944), Zeuner (1946), Stebbins (1947), Rensch (1947), Small (1946, 1948), and others. General agreement seems to have been reached about the slowness of evolution combined with rapidity of changes when they do occur.

Stebbins (1947) suggests that there was an 'evolutionary burst' among angiosperms during Pleistocene 'involving species populations which came together after long periods of isolation in glacial refugia', and that this gave rise to the 'great predominance of polyploids in the glaciated areas' by hybridization and chromosome doubling, allopolyploidy, &c. (cf. Löve and Löve, 1948). Another similar 'burst' is said to be just beginning as the result of hybridizations resulting from the colonization of new habitats being created by human activities of various kinds. Just (1947) summarizes many opinions about 'bursts of production' followed by 'periods of relative stability' particularly among angiosperms.

There need not be any significant parallel developments, comparable with all this Tertiary and post-Tertiary speciation on land surfaces, among the numbers of species of marine micro-floras or micro-faunas, such as diatoms and foraminifera.

Quantitative analyses. Evolution, or change in time, has been slow, and the process of speciation has been relatively rapid. Therefore some actual historical data are very necessary for further progress in our understanding of these processes. The present numbers of species in various taxonomic categories, such as genera, families, or larger groups of species, must have been attained by some arithmetical process or processes. There have been additions and subtractions, as origins and extinctions. On the old views of evolution, which may be described briefly as sympodial, each addition of one or two new species involved the subtraction or extinction of the parent species; but with the new view, of coexistence and monopodial developments, each addition does not necessarily involve a simultaneous subtraction, so that subtractions or extinctions cease to have the direct correlation with origins, which was assumed by Simpson (1944, pp. 17, 20–7, 128, 131–3), following the old general view that one organismal form disappeared because it *changed into* another form. Simpson, however, found that the data for generic durations ('survivorship') in Pelecypoda (bivalve molluscs) demonstrated the presence in his records of two quite distinct types of duration (op. cit., p. 133). The only logical way of investigating the frequency distributions of generic and specific durations is to measure and count such data in a group for which the records are as nearly complete as possible with our present knowledge. This has been done for diatoms (Small, 1945–8) and for the genera of Foraminifera (Small, 1948*b*).

History of present numbers. There have been various theories to account for the present numbers of species and genera. According to the views held by Linnaeus they were all or nearly all attained by a direct process of special creation, and this involved no objective arithmetical development. According to the Darwinian and neo-Darwinian views these present numbers are the result of multifarious causes which have not been separately analysed to any considerable extent and which have therefore given results that may be taken as governed by the laws of probability. The frequency distribution of such numbers should follow various probability curves, and the multifarious causes are regarded as so complex that they cannot all be analysed out arithmetically or quantitatively in any other way.

Sources of data. Arithmetical analyses require some kind of definite arithmetical data, and it is now generally recognized among those who use such data that the most suitable and comparable lists of species and genera are those compiled by one person in a review of one large group. Mills's 'Index to the Genera and Species of the Diatomaceae' (1933–5) has been taken as a fixed book of reference for numbers of valid species and genera of diatoms.

These numbers have been used throughout for arithmetical analyses, but they are not completely definitive, although they are more or less precise and refer to a definite stage in our knowledge of the diatoms. They represent something over 90 per cent. of a complete diatom history, with about 7,000 species listed, and something less than 700 more described since 1935 or likely to be described later. The number of new *fossil* species described for Tertiary deposits is relatively small, but there has been a large increase (about 107 in known upper Cretaceous diatoms (Long, Fuge, and Smith, 1946), and also an extension into Cretaceous of the stratigraphical ranges of 43 species already known in Tertiary deposits. The new fossil species have been described by Hustedt (1935–45), Iyengar and Subrahmanyan (1943), Reinhold (1931–7), Silva (1946), Subrahmanyan (1946), and others.

Fossil diatoms. There is a very large literature dealing with Tertiary deposits of the siliceous shells of diatom frustules. The microscopic complexities of structure make it relatively easy to establish the specific identities of these well-preserved fossils, and new species have been sought by diatomists for over a century. The resulting knowledge is given in a multitude of records which are listed in the bibliography given in Mills's 'Index'. For five years most of my time was occupied with a routine tabulation of geological records for all fossil species of diatoms. By covering all the available literature, and particularly by obtaining much help from the diatomists, F. W. Mills, Esq., and Dr. D. McCall, all the species admitted as valid in Mills's 'Index' were placed in geological time. The main sources of information have been enumerated elsewhere, with the tabulated results (Small, 1945*b*).

Time-scale and deposits. The chronology used includes an initial Cretaceous (C) for relatively few species, followed by Paleocene (Pa); lower, middle, and upper Eocene (LE, ME, UE); Oligocene (O or Og); lower, middle, and upper Miocene (LM, MM, UM); lower and upper Pliocene (LP, UP); with Pleistocene and Holocene taken together as Recent (R). This divides the approximate 70 million years of Tertiary into ten approximately equal subperiods, with a tendency for the Neogene five to be rather shorter than the Paleogene five subperiods (Holmes, 1947, p. 145). For the calculations used here in Tables I and II, and in Figs. 1–4, two additional subperiods of equal length have been introduced for upper Cretaceous, as UC3 and UC4.

The various diatom deposits were placed in these subperiods by following accepted geological opinions, and by careful analyses of Hèribaud's records. The chronology used for named deposits is given in detail as Table III in Small, 1945*b*.

The diatom diagram. From all this routine tabulation of geological records for species of diatoms, very little of interest emerged until it became obvious that species can be classified into two categories with geological ranges or durations which have been either '*short*' confined mainly within part of one subperiod and not extending beyond two adjacent subperiods, or '*long*' mainly from the species origin up to Recent and always covering more than two subperiods. When all the species with Tertiary fossil records were thus classified into evanescent or *e*-species, and permanent or *p*-species which have persisted or tended to go on in time, a large number of interrelated regularities were soon uncovered within the mass of tabulated durations. In fact, it became clear that diatom history was a rather complicated diagram of numerical evolution in a group which was free from most of the disturbing factors of life on a land surface.

These factors include scarcity of inorganic food and for animals organic food, restriction of suitable habitats or ecological niches, glaciation, frosts, droughts, and obligate bisexual reproduction for higher animals as contrasted with the extensive asexual multiplication of diatoms.

Most diatoms are marine, living in large ocean currents as unicellular autotrophic organisms multiplying by vegetative cell-divisions and only occasionally producing reproductive auxospores, by apogamy or autogamy in centric types and some pennate types (Fragilariaceae), and by the allogamous fusion of nuclei from two parental cells in the other pennate families, here distinguished as the allogamous Pennales. There are also many freshwater species, mostly pennate types; and some pennate types which have a raphe and are mobile can exist in moist soil, often as much smaller organisms than their typical aquatic forms (Lund, 1945–6).

The details of diatom history show that centric types were the usual diatoms during early Tertiary, and that there was an increasing predominance of pennate types in late Tertiary, but even now most Centrales are marine diatoms and most freshwater diatoms and all soil diatoms are in Pennales.

The regularities of arithmetical developments in numbers and durations of diatom species have been expounded and summarized in various ways (Small, 1945–8), but this contribution deals with a further analysis of the factors which have contributed to the observed increase of species number in the two main groups, allogamous Pennales and Centrales.

The allogamous Pennales exemplify a relatively young group in which the *initial* phase and the *increase* phase can be very clearly traced and analysed quantitatively, and for which there is only doubtful evidence of an obscure beginning of the phase of *decline*, which is usually part of the detailed numerical structure during the apparently *stationary* phase.

The Centrales, on the other hand, constitute an example of a relatively old group which is passing from the phase of *increase* towards a *stationary* phase within which quite considerable elements of decline can be traced in the histories of single genera.

There is, therefore, some interest in analysing the numerical factors which are similar in type but different in arithmetical values for these two groups. This is no longer an isolated arithmetical curiosity: these analyses show how the various phases in the growth, development, and decline of various taxonomic units can be understood as the result of measurable durations of units and rates of production of units, which are more or less regular in time and which show some irregularities as modifications introduced by external circumstances.

THE ALLOGAMOUS PENNALES

As one very clear example of an *initial* phase followed by a phase of *increase* with multiplication factors for temporary and residual increase of numbers, the history of species-number in all the allogamous families of Pennales taken as one group is very instructive.

Present total. The total number of species in this group, allogamous Pennales, is 3,526 for species occurring in Recent, including Pleistocene. This total refers to species accepted as valid in Mills's 'Index' (1933–5). Several hundreds of new species have since been described, mainly in Recent habitats, so there is no use in laying stress upon the precise number except for arithmetical purposes; see Hendey (1937), Hustedt (1935–45), Ross (1947), &c.

The only species of this group which are recorded for Paleocene are three in the genus *Navicula*, *N. praetexta*, *N. hennedyi*, *N. yarrensis*; all three are known to be now living, and the first two are known from very many deposits throughout Tertiary.

From these initial three species the present total of 3,526 could have been attained in various ways, but the factual history shows an approach to a more or less regular geometric progression, which can be expressed as: final number $= i \times 2^n$, where i is an initial number and n the number of geological subperiods, taken as approximate tenths of the total for Tertiary time (about 70 million years). There are, of course, many other ways of expressing this relation, see Small (1945–9) and examples of normal statistics. The subperiod time scale is only approximately uniform, so that the intermediate values for species-numbers in successive subperiods can be only roughly approximate to any uniform series, even if the actual rate has been completely constant in relation to uniform time.

These approximations are sufficiently precise and regular to show some arithmetical detail, including a distinct and measurable difference between the temporary increases within each subperiod and the overall or residual increases which have contributed to the building up of the present or final total.

Mathematically, the number 3,526 is equal to (*a*) $0{\cdot}8608 \times 2^{12}$, and is also equal to (*b*) $1{\cdot}0000 \times 2^{11{\cdot}7835}$, and both these series approximate to (*c*) $3{,}526 \times 0{\cdot}5^{12}$. Taken to the nearest unit these series are:

(*a*) 1–2 — 3–7 — 14–28 — 55–110 — 220–440 — 881–1763 — 3526;
(*b*) 1–2 — 4–8 — 16–32 — 64–128 — 256–512 — 1024–2048 — 3526;
(*c*) 1–2 — 4–7 — 13–27 — 55–110 — 220–440 — 881–1763 — 3526;
(*d*) (1–2)— 3–3 — 8–20 — 64–185 — 296–316 — 575–443 — 3526.

The fourth series (*d*) consists of the known total numbers of species occurring in each of the subperiods of Tertiary to Recent for allogamous Pennales. This factual series (*d*) fits reasonably well to series (*a*) or (*c*) which are exactly the same as each other except that of '3 or 4' the latter was taken for (*c*) and also 27 for 28 and 13 for 14 (Fig. 1).

Apart from the general approximate fitting, the first two steps are still hypothetical, in brackets, and there is a definite shortage in the known number

(443) for upper Pliocene, and possibly for lower Pliocene. In all diatom records there is this Pliocene gap, because the marine deposits of diatoms for upper Pliocene and in part for lower Pliocene are still unknown since they lie below the Recent deposits at the bottom of modern seas.

Residual increase analysed. Since some of the new species produced in each subperiod are not known outside their subperiod of origin, these species cannot have given rise to the other species which arose in subsequent subperiods. Only those species which continued into subsequent subperiods could be possible parents. In general, only the permanents or *p*-species could possibly have been parental. (*Note*: 'permanent' = tending to go on in time, used of form or things; while 'persistent' = tending to continue in the same course, used mainly of persons, without particular reference to time, except for its technical use in botany.)

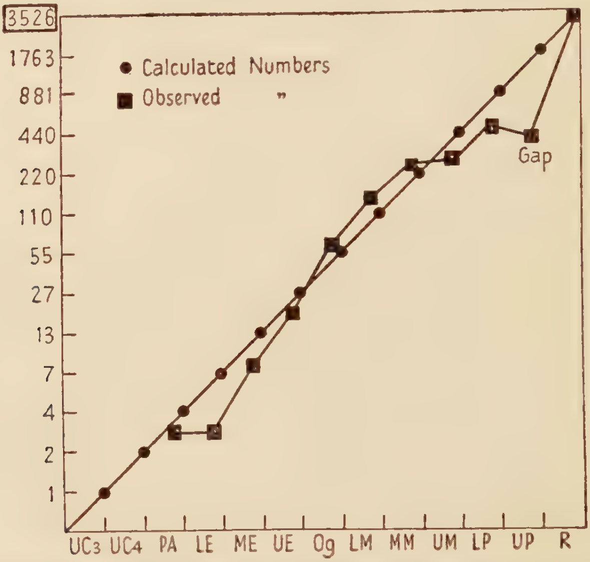

FIG. 1. The total number of species in the allogamous Pennales, occurring in different geological periods (for symbols see p. 95). The calculated numbers assume that the order of increase is regular as given by 1×2^n, where n is the number of periods. The vertical scale is this geometric series.

The residual or overall increases clearly depend upon the numbers of old *p*-species which, in each subperiod, have come on from previous subperiods. The rates of *temporary* subperiod increases can be measured as new/old or *A*/*O*, as for production rates in any taxonomic categories (Small, 1948*b*), using the numbers of new species and numbers of old *p*-species known in any given subperiod.

For allogamous Pennales the values for *A*/*O* during Tertiary have been as follows, starting with an initial lag of two zeros for no observed increase in Paleocene and lower Eocene: 0, 0, 1·0, 1·06, 4·31, 2·38, 1·47, 1·45, 1·39, 0·27, 6·13; which averages out at 1·77 (Small, 1948*a*, p. 300). This 1·77 is the average *temporary increase* per subperiod, therefore the total including the original parents would be 1+1·77 or ×2·77 for each average subperiod. But this rate of increase, if continued even only from middle Eocene, would have given, from the original 3 species, 7,820 in upper Pliocene and 21,660 in Recent, so that some reduction in the rate of increase must have occurred to give an approximation to ×2·0 per subperiod for the observed increase.

The reduction of the observed temporary to observed residual increase might have been applied irregularly or regularly, by intermittent cataclysms as suggested by Yule (1924) or by extinctions due to less regular catastrophes: but the observed residual increases have been more or less regular as can be seen by their fitting to a $\times 2^n$ diagonal in Fig. 1 and by comparing the (*a*) or (*c*) series with the (*d*) series given above. Increase has, historically, proceeded as 1·7 steps forward (temporary increase) followed by 0·7 steps backward with the disappearance of short-lived species in each subperiod, leaving a residue of 1·0 steps forward as the new *p*-species continued (residual increase).

The numerical data for Fig. 1 are given in Table I. The ratio of 9 old *p*: 9 new *p*: 7 new *e*, as the composition of the total species in any one subperiod, means that there will be 18 *p* to 7 *e* for each 25 species occurring. On this basis there should be $\frac{18}{25}$ths of 3,526 (or 2,540) *p*-species among the total occurring for Recent in allogamous Pennales: and since new *p*-species have arisen from old *p*-species in the proportion of 9 new from 9 old, the total number of *p*-species can be calculated for each Tertiary subperiod as a decreasing series from $2{,}540 \times 0{\cdot}5^n$. This series (*a*) and the numbers of *p*-species known for the corresponding subperiods (*b*) run as follows:

(*a*) 2,540 — 1,270 — 635 — 317 — 158 — 79 — 40 — 20 — 10 — 5 — 3 — 2 — 1
(*b*) (441) — (442) — 445 — 280 — 196 — 134 — 36 — 15 — 7 — 3 — 3 — ? — ?

Because *p*-species are defined by their geological ranges, the first two items include only old *p*-species; then 445 for lower Pliocene and earlier subperiods fit reasonably well, on the whole, down to the original three species of *Navicula* in Paleocene. The fitting shows quite clearly in Fig. 2, where the diagonal is the series (*a*) above (final number $\times 0{\cdot}5^n$), and the line with black squares is the observed totals for *p*-species in each subperiod, series (*b*) above.

The totals for *p*-species can be broken up into two halves corresponding to the 9 old: 9 new. The *e*-species also can be analysed out as $\frac{7}{25}$ths of each subperiod total, and this series also is, mathematically, a decreasing $\times 0{\cdot}5^n$ series; but, again by definition, the *e*-species among the Recent total cannot be distinguished, and again there is the Pliocene gap in our knowledge particularly of *e*-species for upper Pliocene. These three series of calculated and observed numbers do not fit so closely as those in Figs. 1 and 2, but they are set out with the others in Table I for reference.

Thus, with a subperiod production-rate averaged at 1·77 for temporary increases in numbers of species, and with an *e*/*p* ratio of 7/9 for reduction of these temporary increases by $\frac{7}{16}$ths as the proportion of species which have

TABLE I

Data for Allogamous Pennales

Geological period	Calculated species-number*					Observed species-numbers				
	Total *OC.*	Total *p*18:	Old *p*9:	New *p*9:	New *e*7	Total *OC.*	Total *p*	Old *p*	New *p*	New *e*
Recent	**3,526**	2,540	1,270	1,270	986	**3,526**	441	441	—	—
Upper Pliocene	1,763	1,270	635	635	493	443	442	437	5	1
Lower ,,	881	635	318	317	246	575	445	280	165	130
Upper Miocene	440	317	158	159	123	316	280	191	89	36
Middle ,,	220	158	79	79	62	296	196	134	62	100
Lower ,,	110	79	40	39	31	185	134	36	98	51
Oligocene	55	40	20	*20	15	64	36	15	*21	28
Upper Eocene	27	20	10	10	7	20	15	7	8	5
Middle ,,	13	10	5	5	3	8	7	3	4	1
Lower ,,	7	5	3	2	2	3	3	3	—	—
Paleocene ,,	4	3	2	1	1	3	3	3	—	—
Upper Cretaceous 4	2	2	1	1	—	—	—	—	—	—
,, ,, 3	1	1	—	1	—	—	—	—	—	—

* Calculated from total *OC.* observed 3,526 R, assuming regularity of the ratios given at the head of each column.

not continued beyond their subperiod of origin, there has been a clear approximation to a $\times 2^n$ residual increase which, arithmetically, is the result of the arithmetical processes summarized as:

$$9 \text{ old} + (16 \text{ new} - 7\ e) = 9 + 9 \text{ or}$$
$$\times[1 + (16/9 \times 9/16)] = \times 2 \text{ per subperiod,}$$

as the common ratio of the geometric progression occurring in the residual increase of species-number in allogamous Pennales.

The additions of new species, and the subtractions of evanescent *e*-species, and the consequential geometric progression or multiplication of permanent species, have all been approximately regular throughout uniform time. This, as has been pointed out, refers to a group in its *phase of increase* during which the number of new species arising is a function of the number of old species continuing in the living condition as possible parent-species.

Rate of increase. The rate of increase in numbers of species among the allogamous Pennales is higher than that for Centrales, but it is a very low rate. The ratio of 16 new from 9 old species per subperiod of about 7 million years means an average increase of one new from one old species in $\frac{9}{16}$ths of 7 m.y. or about 4 million years. Since gradual genetic changes of populations would have made chaos of the diatom fossil record, the actual process of change must have been very rapid with intervals of complete fixity for about 4 million years between each rapid change in any one lineage. These rapid changes left the parent species to continue unchanged, so the change could have affected only a part of the current population.

The simplest way to meet *all* the known facts is to regard each speciation as a one-step change in one organism of the parent species. Mayr (*in litt.*)

agrees that 'asexual reproduction is conducive to evolution by sudden steps, instantaneous speciation', and there is abundant asexual multiplication of unit frustules among all diatoms, which could preserve purity of the new lineage after any specific mutation.

The allogamous Pennales is an example of how the arithmetic of evolution, as change in the numbers of species, has in fact proceeded during the *initial* or lag phase and the *increase phase* of group development. There is some, but very little, evidence in generic details that this group is now approaching its stationary phase after a period of increase which began in upper Eocene about 50 million years ago, and this would agree with Zeuner's views about the length of the 'explosive' phase of evolution within a group of any taxonomic category (Zeuner, 1946*b*, p. 248; see also below).

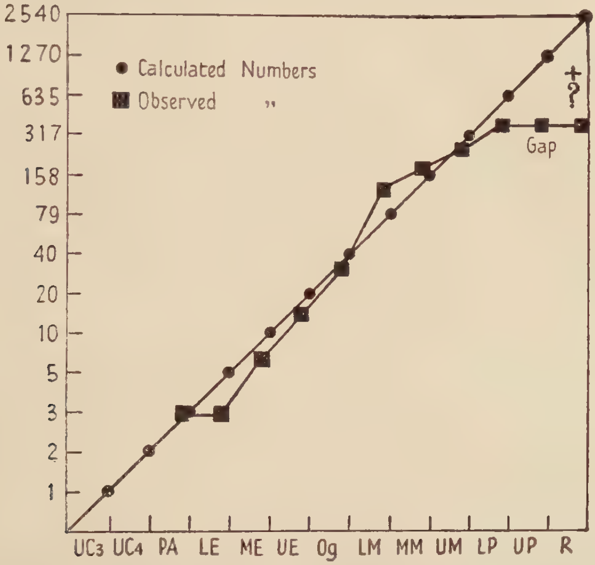

FIG. 2. The total numbers of permanent or *p*-species in allogamous Pennales at different geological periods (see p. 95 for symbols used). The vertical scale is based on the geometric series 1×2^n, where n is the number of periods.

In order to appreciate the significance of this analysis it should be noted that the component factors have been 'observed' separately in the detailed data, and the more or less constant value of the resultant factor is derived by simple multiplication and the addition of 1·0 as the parental proportion of the total number occurring; thus, $1+16/9$ for temporary increases, and $16/9\times 9/16$ for proportion of *p*-species continuing.

The fitting of the factual observed numbers to the calculated diagonals, in Figs. 1 and 2, leaves little doubt that the main factors have been analysed out. These are (1) a more or less constant proportion of new species, the number of new species having been controlled by the number of old *species* available as parents and consequently not by any other characteristic such as number of individuals per species or number of generations per species, or variations in external factors; and (2) a more or less constant proportion of species which have continued into subsequent subperiods, the actual numbers of such species having been controlled by the total numbers of species produced (*a*) in each new subperiod *quota* and (*b*) in every previous subperiod quota.

The large degree of regularity observed in the functioning of these characteristics, during the Tertiary history of allogamous Pennales, is a demonstration that these characteristics are innate genetic factors which have functioned undisturbed, or at least with comparatively little interference from factors outside the organisms concerned. Both species-durations and the distribution of duration types have been inherited characteristics, and so also has been the degree of specific mutability as measured by the subperiod speciation rate.

Further implications. Zeuner (1946*a*, chap. xii; 1946*b*) in his discussions of biological evolution in relation to time, has assembled many examples of various numerical phenomena, such as absolute ages of various groups of diverse taxonomic categories; these ages in millions of years (m.y.) vary from 1 m.y. to 500 m.y., mostly according to the origins rather than completed durations because nearly all the groups given by him have continued into Recent. Zeuner (loc. cit.) also gives many examples of numerical histories in which there have been (1) a relatively insignificant *initial or lag phase*, (2) an *explosive phase* of rapid increase 'which is suggestive of a logarithmic increase' (op. cit., p. 356), (3) a climax or *stationary phase*, and (4) a '*phase of decline* which may be sudden or slow'.

The first two phases indicate 'an underlying exponential function or an increase per unit which is determined by a certain constant factor of multiplication', as Zeuner puts it (op. cit., pp. 357–8). This, it should be noted, introduces an element of multiplication into the arithmetical history, and also the extra idea that the number of new units has been determined by the number of old units. The significance of this arithmetical phenomenon during the phase of increase is fundamental, as it means there is no 1 : 1 correlation of origins and extinctions.

The arithmetical value of the multiplication factor varies from period to period within relatively narrow limits in some cases. Zeuner has expounded the 'explosive' phase of increase in tables and in time-frequency diagrams for species in the genus *Salenia* (a sea-urchin), for species in the family Clypeastridae (sea-urchins), and for genera in the family Spiriferidae and the superfamily Spiriferacea (Brachiopoda). He gives total numbers of units as linear magnitudes, and also in histograms as block-areas against uniform basal time-scales, so that curve-forms rather than actual data are presented.

Small (1948*a*) has given for diatoms the actual arithmetical data grouped in various ways and plotted against an approximately uniform time-scale.

New units cannot arise from old units which are extinct at the time of the origins of these new units, so the old units which are not extinct at the time of origin of new units are the only possible parents for new units in whatever taxonomic category the units are taken. Then the number of new units arising (A) is proportional to the number of persisting old units (O) in a ratio which is measurable as A/O; this gives a measure of the rate of production of new units for any given section of time. This ratio, as $1+A/O$, is a measure of the multiplication factor or the rate of increase from point to point along a uniform time-scale for the whole duration of groups of units. It has been so used for speciation-rates of the genera and of the main groups of diatoms (Small, 1946 and 1948*a*), also for the production-rates of families and genera in foraminifera, of genera and species in Polymorphinidae (foraminifera), also genera and species in the two main groups of diatoms (Small, 1948*b*).

Among taxonomic units of upper Cretaceous or Tertiary origin, the first two phases, *initial* and *increase*, may be the only phases present in the records; but among units with earlier origins the data may include the third or *stationary phase* and possibly also the fourth or phase of *decline*. It is now possible to analyse out various factors which contribute to the production of these various phases, particularly in the history of the diatoms.

According to Zeuner (1946*b*, p. 248) the phase of *increase* or 'explosive phase' has commonly lasted for about 50 million years, and this length has been quite independent of the taxonomic category or kind of organisms considered. It has been of the same order of duration for species, genera, and orders: also, 'The significance of this apparent limitation of the duration of explosive phase is still obscure.' He suggests that it may mean that 'the number of species-steps involved in the evolution of a new family or order is not greater than that involved in the evolution of new genera'. Small (1948*b*) has shown that the origin of all new units of whatever taxonomic category can be traced to one new change which may be one of various qualities, the quality of the change determining the taxonomic category of the new specific unit, as suggested by Sewertzoff and by Goldschmidt (1940) (see Zeuner, loc. cit.).

THE CENTRALES

As an old group, with a few of its earlier large genera now much reduced in living species and a few now large genera which have produced only a few *p*-species since middle Miocene, the Centrales shows signs of the action of the same component factors as have been distinguished in the data concerning the allogamous Pennales. Nevertheless there are various large differences due to relative age, to the presence of an element of decline, and of different arithmetical values for the two main factors of overall increase.

Present total. The total number of species in this group, Centrales, is 1,438 for species occurring in Recent, including Pleistocene. This total refers to species in Mills's 'Index' (1933–5) and, as mentioned earlier, only a few dozen new species have since been described for Tertiary and Recent, although 107 new species have been described from the pre-Tertiary Cretaceous deposits at Moreno Gulch.

In the case of the allogamous Pennales the *initial* or *lag phase* is present in the Tertiary history of the group, as the initial 3 *p*-species for Paleocene and lower Eocene, but with the Centrales there was at least one large generic complex, *Hemiaulus-Trinacria,* which had reached its maximum in or before Paleocene, so that here we are dealing with an old group which had probably a long pre-Tertiary history before the arithmetical phenomena of its Tertiary history were developed.

Overall increase analysed. In Centrales the proportion of evanescent or *e*-species in the Tertiary subperiod quotas of new species has been demonstrated as having been 3 *e*-species to 1 *p*-species. This 3/1 *e*/*p* ratio has been proved as having been maintained throughout the Tertiary subperiods for Centrales as a group, for many centric genera each as a whole, and in generic subperiod quotas of new species (Small, 1945*a*, 1946, and 1948*a*, pp. 265–75).

This large proportion of *e*-species, three-quarters of the total of each subperiod quota, makes it rather more difficult to use the total number of species occurring in any one subperiod as a suitable basis for detecting any underlying regularities in the component factors of increase. The Pliocene gap is almost certainly larger for centric species than for pennate species, because more species of Centrales are exclusively marine. Some of the pre-Tertiary fossil *e*-species are probably mixed up with the Paleocene records, many of which are from submarine deposits near Franz Josef's Land. The Moreno Gulch Cretaceous deposits were at first said to resemble closely some of the Russian deposits which have been taken as Paleocene (Hanna, 1927; Small, 1945*b*, p. 297), so the records for the Russian deposits, especially the submarine ones, may well include a fossilized accumulation of *e*-species from pre-Tertiary times. There are also old submarine deposits of diatoms along the west coast of Africa, between Dakar and Walfish Bay, which very probably include many pre-Tertiary species, but the analysis of Leuduger-Fortmorel's records (1898) has been a long and complex piece of work quite beyond the scope of this present contribution.

With all these irregularities and uncertainties the *e*/*p* ratio of 3:1 stands out as clearly demonstrated in considerable detail (Small, 1948*a*, pp. 265–75). This was the first regularity to be uncovered among the diatom data (Small, 1945*a*) and was the key to all the other regularities. The maintenance of this ratio of 3 short-lived or *e*-species to 1 long-lived or *p*-species in most of the centric genera, in all the subperiod quotas and in the group as a whole (1,230:403), made various other analyses possible.

The analysis of subperiod speciation rates in allogamous Pennales was carried out above by averaging the *group* rates, but in the case of Centrales there are several factors, such as decay of speciation in some genera, which have led to a better analysis by other methods of approach.

Each one of the 351 generic subperiod speciation rates has been set out for all three main groups of diatoms (Small, 1948*a*, p. 305), and, when those for Centrales are examined and grouped (op. cit., p. 307), it is found that the distribution of these rates is such that there are 43 values up to 0·45, 80 between 0·46 and 1·82, with 32 at 1·0, and 60 above 1·83. Since there has been a mode somewhere around 1·0, and since this subperiod speciation rate yields the best fitting of detailed facts to *generalized* residual increase data, the analysis of these data has been based upon this value.

The *temporary* subperiod increases can be measured as A/O or new/old species in each subperiod. They have been, starting with lower Eocene, as follows: 1·12, 0·8, 3·68, 1·13, 1·48, 0·97, 0·52, 1·14, 0·14, 3·77; which averages out at 1·475 per subperiod for Centrales, instead of 1·77 as for allogamous Pennales (Small, 1948*a*, p. 300). In all subperiods, except middle Eocene, the pennate subperiod production rates for species have been higher than the corresponding centric production rates to the extent of $\times(2{\cdot}0\pm0{\cdot}8)$, but since these are logarithmic characteristics the consequential values do not have straight arithmetical relations. The extreme values for subperiod production rates in the two groups AP/C are 0·27/0·14 and 6·13/3·77, and these yield ratios of 1·9 and 1·6, average 1·75, which, with the allogamous Pennales increase being about 1·77 per subperiod, again yields about 1·0 for the corresponding temporary subperiod increase in Centrales (Small, 1948*a*, p. 300).

The average value of 1·475 for *group* production rates is too high to fit the residual increase data, but if the modal value of 1·0 for generic subperiod production rates is taken as a basis, this can be combined with the known proportion of *p*-species (1/4). Adding the parental 1·0, the answer to this sum is: $1+(1\times1/4) = 1{\cdot}25$, as a numerical value for the residual increase of species number in Centrales, as compared with $1+(16/9\times9/16) = 2{\cdot}0$ for allogamous Pennales.

This 1·25 is the common ratio (r) of a rather slowly rising geometric progression: it gives the 'slope' for rectilinear diagonals corresponding to those for allogamous Pennales in Figs. 1 and 2, where r is 2·0. But the problem of fitting involves choosing a suitable starting-point. For the allogamous Pennales the value of 1 could have been taken, but the final (present) value of 3,526 was in fact taken and divided by 2·0 to give a reducing series which, of course, is much the same as starting with a hypothetical 1, and applying the lowest known number of 3 to the corresponding point on the mathematical diagonal.

In the case of Centrales, the suitable known number could not be anything like 3, because of the long pre-Tertiary history of this group, and the earliest total, that for Paleocene, is suspected to contain a pre-Tertiary element of *e*-species. The Paleocene *p*-species are not affected by this uncertainty because their subsequent Tertiary history proves them as permanent, and whether they began in Cretaceous or in Paleocene does not affect their status as valid *p*-species known in Paleocene. The number of *p*-species in Paleocene (91) was, therefore, taken as a suitable point for the application of the observed data to the diagonal calculated for *p*-species.

With this point of application settled, the calculated distribution of *p*-species throughout Tertiary becomes a regular geometric progression in which each successive term is $(\times1{\cdot}25)^n$, following the formula for residual increase $1+(1\times0{\cdot}25)$ but using only *p*-species, and starting with 91 in

Paleocene as equal to ×(1+0·25), giving 73 old and 18 new *p*-species. This series, from Paleocene onwards, for total *p*-species in the succession of subperiods (*a*) can be compared to the observed series for the same subperiods (*b*) as follows:

(*a*) 91 — 114 — 142 — 177 — 221 — 276 — 345 — 431 — 539 — 674 — 842;
(*b*) 91 — 105 — 119 — 203 — 228 — 273 — 309 — 269 — 280 —(259)—(259).

By the definition of *p*-species, at least the last two items of series (*b*) cannot be fitted because the necessary durations are not known for species which began in upper Pliocene and Recent. The same might be said to apply to lower Pliocene, but there is also a deficiency of species with LP-R and UM-R records. This is a real phenomenon in the records, which may be in part an extension of the Pliocene gap in these records for mainly marine genera: it is also due in part to a decrease in subperiod speciation or to a smaller than normal increase in subperiod speciation among genera which ceased to expand in or before upper Miocene. The detailed facts can be followed in the basic tables of generic histories (Small, 1945*b*, Tables I and II).

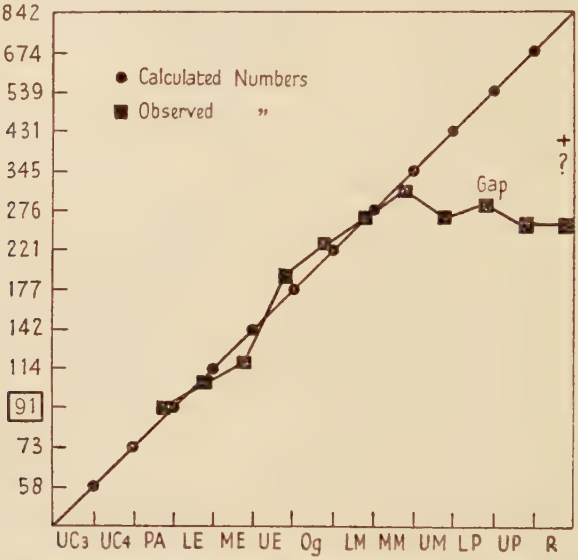

FIG. 3. Total permanent or *p*-species of Centrales in different geological periods (for symbols see p. 95). The vertical scale is a geometric series described in the text.

Starting with 91 *p*-species in Paleocene, using the factor ×1·25 per subperiod for *p*-species only, the (*a*) and (*b*) series for calculated and observed numbers can be graphed as in Fig. 3, where the fit of the observed numbers is seen to be reasonably good from Paleocene to middle Miocene, after which the decline in production of *p*-species in most large genera combines with the deficiency due to the definition of *p*-species in the appearance of a gap between observed and calculated numbers. This 'gap' thus has a double origin, (1) the impossibility of assessing the duration from UP to R or in Recent-only as 'long', and (2) a recognizable decrease in *p*-species production by many genera for lower Pliocene and upper Miocene. The second factor is a recognizable element of the *decline phase*, and another aspect of the same decline is to be traced in the extinctions during middle Miocene of species which have been long-lived: there were 46 of these extinctions in MM, 17 in UM, and 23 in LP (Small, 1946, fig. 4, p. 61). But sufficient long-lived species continued, in the now large genera, to give from their accumulated numbers the known larger total numbers of species found only in the Recent.

There has been as yet no similar decline in allogamous Pennales, where only 5 genera and 14 *p*-species have become extinct, as compared with 61 genera and 144 *p*-species now extinct in Centrales (Small, 1946, figs. 2 and 4).

Centrales subperiod totals. Bearing in mind the various degrees to which old genera in Centrales have passed into generic *phases of decline* especially in production of new *p*-species in late Neogene (UM to UP), and also the generic maxima for *e*-species shown by some large genera for Paleocene, upper Eocene, and middle Miocene, no great regularity can be expected within the relatively small residual increase for *total number* of species, which has occurred over the ten subperiods of Tertiary time and into Recent. But these observed numbers can be compared as a series (*b*) with a calculated series based first upon an increase of *p*-species from 91 in Paleocene by ×1·25 per subperiod. It is necessary next to add ⅗ths of the number so obtained to each subperiod quota as the proportion of *e*-species which would be given by the distribution of each subperiod quota in the proportion of 4 old *p*-species to 1 new *p*-species and 3 new *e*-species. This 4 old *p*:1 new *p*:3 new *e* for Centrales contrasts with 9 old *p*:9 new *p*:7 new *e*-species for allogamous Pennales. The distribution of new species as 1 new *p*:3 new *e* is in accordance with the *e*/*p* ratio of 3/1 which has been observed for subperiod quotas of new species in the Centrales as a whole (Small, 1945–8).

These two series of numbers, (*a*) calculated and (*b*) observed, for the total numbers of Centrales in the subperiods Paleocene to Recent are:

(*a*) 146 — 182 — 228 — 284 — 354 — 442 — 552 — 690 — 862 — 1,078 — 1,348;
(*b*) 361 — 132 — 127 — 356 — 372 — 426 — 523 — 299 — 359 — 260 — 1,440.

The fit, from the second item (lower Eocene) to the seventh item (middle Miocene), is better than might have been expected. The deficiency in middle Eocene, 127 for 228 calculated, is certainly due to our lack of knowledge since the *e*-species for that subperiod are known only from the Kreyenhagen shales (Hanna, 1931), assuming these do belong to that subperiod. The 'excess' for Paleocene, 361 for 146 calculated, almost certainly includes a large admixture of pre-Tertiary *e*-species from submarine deposits allocated to Paleocene, and quite possibly other Cretaceous *e*-species from the lower parts of the Russian deposits, which have been classed as Paleocene but closely resemble the material from Moreno Gulch, which is now very definitely placed at the top of Upper Cretaceous (Hanna, 1927; Long, Fuge, and Smith, 1946). The point for 361 in Paleocene is marked and isolated in Fig. 4, where the above two series (*a*) and (*b*) are graphed against the usual subperiod time-scale.

The large difference, between the upper Eocene with its included generic maxima and the middle Eocene (ME) which is known to be minimal in our knowledge of its *e*-species, lies across the diagonal, but a direct connexion of the LE point to the UE point would present a reasonable fitting. It must be remembered that these numbers differ very little, as compared with the $\times 2^n$ progressions in Figs. 1 and 2, and also that here we are dealing with a very mixed collection of genera in different *phases* of generic development. The extensive gap from upper Miocene (UM) to upper Pliocene (UP) corresponds closely with the similar gap for *p*-species, which has already been discussed above and illustrated in Fig. 3. The problems raised by these divergences are slightly different, but the deficiencies again have a double origin: (*a*) there is still the upper Pliocene gap in our knowledge, particularly of marine species which began and ended in that subperiod, that is, of upper Pliocene *e*-species which lie below the Recent deposits of diatoms at the bottom of existing oceans; and (*b*) *e*-species are derived from *p*-species and it has been shown in the above discussion of *p*-species that production of *p*-species declined in Centrales during late Neogene (UM-UP), so that parents for *e*-species did not increase during that time in the same proportion as they did during the early subperiods of Tertiary. From (*a*) and (*b*) in the above sentence the difference between the calculated and observed numbers for total species might be explained: the facts of history for Centrales seem to have differed from a

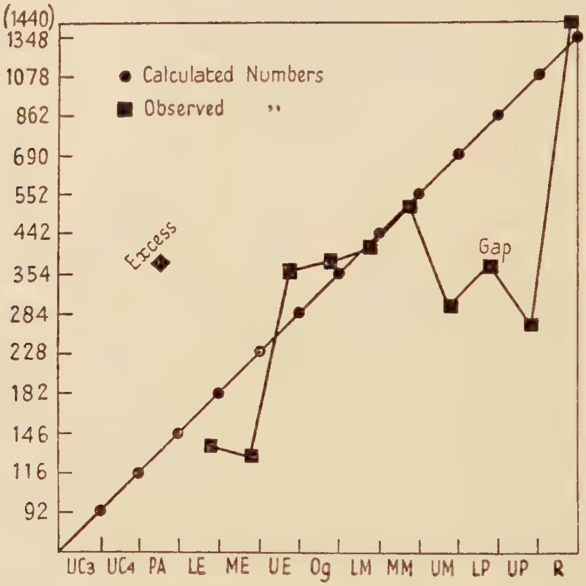

FIG. 4. Total numbers of species of Centrales occurring in different geological periods (for symbols see p. 95). The vertical scale is a geometric series described in the text.

plain geometric progression continuing beyond middle Miocene into Recent as was found for allogamous Pennales. These facts include extensive *incipient* decline in old genera, which shows as a decrease in the production of new *p*-species, particularly in late Neogene (UM-UP).

But the above explanations of the UM-UP gap seem to prove too much, because the same calculated series with its continued increase for total species-number arrives at 1,348 for Recent, which is a much closer approach to the 1,440 observed than any of the late Neogene values: and this 1,348 can be *calculated* as consisting of 674 old *p*-species, 168 new *p*-species, and 506 new *e*-species, from the formula for distribution—4 old *p*:1 new *p*:3 new *e* (see Table II below).

This raises another problem, one solution of which could be suggested to be that the late Neogene decline in production of *p*-species may apply only to certain very old genera and that the younger genera have compensated for this decline by producing enough *p*-species to maintain the general residual increase in all three types of species. The younger genera with known Tertiary records have not done this, but there are 38 centric genera which had only Recent records in the original analysis.

Another aspect, which definitely rules out this suggested solution, is that all the Recent-only (Ro) genera taken together include only about 89 species, so that they could not account for the difference of about 900 species between the 1,440 for Recent and the 523 for middle Miocene. Among the 38 Ro genera in Centrales there are 18 monotypes, 8 ditypes, 3 tritypes, 2 with 4 species, and one each with 5, 6, 7, 10, 11, 13, and 23 species. Allowing for recently found fossil records which transfer one of the tritypes, the 11-type, and the 23-type to the fossil-and-recent or FR class; this represents a total of 89 species in 35 Ro genera. The small size, of 1–4 species with a limit about 8, is characteristic of genera with a duration which has not been long, whether these genera are Recent only or short-lived extinct genera (Small, 1948*a*).

The numerical facts are that, to our present knowledge, the total number of Centrales reached 523 in Middle Miocene, varied through 299 UM, 359 LP, 260 UP, and rose to 1,440 in Recent; but in view of the regularities found for allogamous Pennales throughout Tertiary and for Centrales *p*-species from Paleocene to middle Miocene and for the total centric species from lower Eocene to middle Miocene, our present knowledge may not be the complete truth.

The only logical solution of this problem, which accounts for the 1,348–1,440 total species in Recent, is that we know only part of the late Neogene diatom flora for *old* genera, and that the remainder still lie hidden below the Recent diatom deposits at the bottom of the present oceans. This view is supported by the known upper Miocene and lower Pliocene quotas of new species, which quotas show approximations to a 3:1 *e*/*p* ratio thus: UM 30/6 and LP 79/28, which have deviations of only +3 and −1 from nearest approaches to 27/9 and 80/27 with totals of 36 and 107 respectively.

The argument here is that among the known species, as subperiod quotas for these two subperiods, the main ratio is approximately maintained; therefore we know neither all the *e*-species, nor the earlier than Recent records for many of the *p*-species which presumably originated in upper Miocene and lower Pliocene and now contribute to the known total of species which occur in Recent. The *e*-species would have begun and ended in these two subperiods, and do not now occur in the records of Recent species; but the *p*-species would have continued into Recent and given off their quota of new *p*-species and new *e*-species. All these must still be labelled Ro in the absence of fossil records for both parents and progeny.

TABLE II

Data for Centrales

	Calculated species-numbers*					Observed species-numbers				
Geological Period	Total *OC.*	Total *p*5:	Old *p*4:	New *p*1:	New *e*3	Total *OC.*	Total *p*	Old *p*	New *p*	New *e*
Recent	1,348	842	674	168	506	1,440	259	259	—	—
Upper Pliocene	1,078	674	539	135	404	260	259	257	2	1
Lower ,,	862	539	431	108	323	359	280	252	28	79
Upper Miocene	690	431	345	86	259	299	269	263	6	30
Middle ,,	552	345	276	69	207	523	309	255	54	214
Lower ,,	442	276	221	55	166	426	273	210	63	153
Oligocene	354	221	177	*44	133	372	228	184	*44	144
Upper Eocene	284	177	142	35	107	356	203	116	87	153
Middle ,,	228	142	114	28	86	127	119	105	14	8
Lower ,,	182	114	91	23	68	132	105	91	14	27
Paleocene	146	**91**	73	18	55	361	**91**	73?	18?	270
Upper Cretaceous 4	116	73	58	15	43	—	—	—	—	—
,, ,, 3	92	58	46	12	34	—	—	—	—	—

* Calculated from total *p*-species 91 in Paleocene, assuming regularity of the ratios given at the head of the columns. (See text.)

This should give an added interest to the examination of modern deep-sea cores which may penetrate the thick layers of modern deposits, and so reach down to the Pliocene and upper Miocene layers assumed to lie below these modern deposits. Deep-sea cores taken off the west coast of Africa, between Dakar and Walfish Bay, would probably have a still wider significance by penetrating through, or past, many Tertiary layers to pre-Tertiary deposits which have remained entirely submarine since the beginning of Cretaceous. These older beds seem to be exposed in some places, as if the strata lie tilted, below the sea. See Lohman (1941) for core technique.

Divergence from recognized rules or regularities always have special interest, particularly when they lead to further searches for explanations, either immediately or in the future. In the case of the divergences found in Fig. 4, immediate searches have disclosed the possible types of record which would tend to fill up the obvious gap, but the way is opened for future work which might remove the divergences altogether. The kinds of records to be expected from the examination of deep-sea cores are clearly indicated, namely, (*a*) a number of new fossil records for species now known only as Recent, and (*b*) about three times that number of records for species not now known at all.

The 'expected' numbers of species in these two categories are set out with the other data for Centrales which have been discussed above, in Table II. The divergences of the observed and calculated numbers for Centrales make this Table II less of a direct arithmetical demonstration of regularities than the Table I and Figs. 1 and 2 for allogamous Pennales; but the presence in the Centrales data of similar regularities and of divergences from these regularities are demonstrated by the above detailed analysis.

SUMMARY

1. The phenomena of quantitative evolution in diatoms are discussed in relation to the new outlook on evolution as shown in some recently published books and papers, particularly since 1941; and a large amount of agreement in general and in detail is demonstrated.

2. Just (1944) concluded his discussion thus: 'The rates of evolutionary processes differ so greatly that they can not be expressed in simple mathematical terms.' This is very true, and numerical analyses show a number of correlations which are interconnected in a complex manner.

3. Residual or overall increase in numbers of species is here shown to be correlated with the following:

(*a*) the phases of development of a group, and of its constituent genera;

(*b*) a rate of increase which has been more or less uniform during the *increase phase*, and which has been correlated with and controlled by—

(*c*) the rate of production of new species; and

(*d*) the proportion of new species which have been of the permanent type.

4. The factors 3 (*c*) and 3 (*d*) have been measured, and correlated with their resultant as 3 (*b*), in both the main groups of diatoms, thus:

For allogamous Pennales the speciation rate has been a proportion of about 16 new species from 9 old species (or 1·77) per subperiod of about 7 million years, and the proportion of permanents or *p*-species has been about 9 out of 16.

For Centrales the speciation rate has been a proportion of about 1 new species from 1 old species (or 1·0) per subperiod of about 7 million years, and the proportion of permanents or *p*-species has been about 4 out of 16.

5. There has been a marked difference in the arithmetical history of the two groups during Tertiary time, and in the end-products of these two histories.

6. Four phases of development of a group or genus are recognized, namely, an initial *lag phase*, an *increase phase*, a *stationary phase*, and a *decline phase*. The allogamous Pennales shows only the first two phases, and the Centrales has approached the *stationary* phase as a group, while showing all four phases in some of its constituent genera.

7. The end-products of the history of Centrales, with its many extinct genera and its elements of the *decline phase* in other old genera, in the form of the frequency distribution of generic sizes, are similar to those of some other groups of plants and animals.

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The Perithecia of *Ophiostoma Majus* (van Beyma) Goidànich

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With six Figures in the Text

INTRODUCTION

ACCOUNTS of perithecial formation in the genera *Ophiostoma* and *Ceratostomella* have been given by Saccardo (1882), von Hohnel (1918), Sydow (1919), Dade (1928), Nannfeldt (1932), Nannfeldt and Melin (1934), Davidson (1935), Andrus and Harter (1933, 1937), Andrus (1936), Goidànich (1935), and Gwynne-Vaughan and Broadhead (1936). These accounts suggest that there are fundamental differences between species in both genera and they differ in the interpretation of observations based on *C. fimbriata.* These differences are related mainly to the method of division of the ascogenous cells, the formation of asci, and the cleavage of ascospores, there being general agreement in the accounts of perithecial wall and neck formation. The present detailed examination of *O. majus* was undertaken since the method of perithecial formation differs in important details from that in other species under examination, or in published descriptions. Descriptions of the species under investigation will be published later. A detailed examination of all species of the two genera may indicate more satisfactory criteria for the determination of natural relationships than the characters of the perithecial wall and shape of the ascus on which the present classification is based.

MATERIALS AND METHODS

The strain of *O. majus* isolated from beech sawdust and used for a previous investigation (Hutchinson, 1939) was examined. Stock cultures were kept on prune agar slopes at room temperature, and at first were subcultured at monthly intervals. After 6 months fruiting became erratic and smaller perithecia were formed. Examples of these fruiting and non-fruiting cultures were then subcultured at intervals of 7 to 10 days. After 6 months of this frequent subculturing there was a noticeable increase in the number and size of perithecia formed. After 12 months production was abundant, and the perithecia formed were of similar size to those in the first isolations. Table I compares the size of the perithecia in the original isolations, those formed 6 months later, and the measurements given by van Beyma in the original description (1935), which was based on culture material. The Centraalbureau

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voor Schimmelcultures have compared cultures of this strain with the type culture of the species, and agree that it is *O. majus*.

Table I

	Strain under examination.		
	Measurements shortly after isolation.	Measurements after 6 months in culture.	Measurements by van Beyma of type culture.
Diameter .	600–1,000 μ	350–450 μ	320–480 μ
Neck . .	2·5–4·5 μ × 60–95 μ	2–3 μ × 60–70 μ	2–4 μ × 50–80 μ
Ostiolar hairs .	100–250 μ × 3–4 μ	200–250 μ × 4 μ	250–325 μ × 4 μ
Ascospores .		9·5–7·1 μ × 5·9–4·1 μ	7·3–8·3 μ × 3·4–4·7 μ

Material for examination was obtained from petri-dish cultures on prune agar kept at 27° C. These were examined at intervals, and portions of agar bearing the required stages cut out, fixed in Fleming's weak solution, and embedded in paraffin wax of m.p. 57° C. by use of cedar-wood oil. If softer waxes were used the brittle perithecial wall tended to fall apart during sectioning. Sections were cut from 2 to 7 μ in thickness and stained with Haidenhain's iron-alum-haematoxylin, which gave satisfactory preparations of older stages when used alone. Younger stages were counterstained with erythrosin in 90 per cent. alcohol, orange G in water, or light green in clove oil, erythrosin giving the clearer preparations. Gentian violet was used for the examination of the walls of the fertile cells.

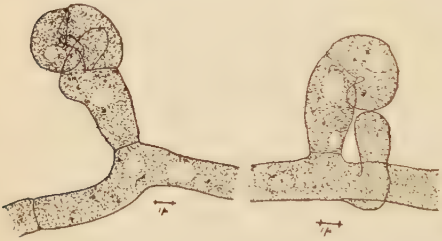

FIG. 1*a*

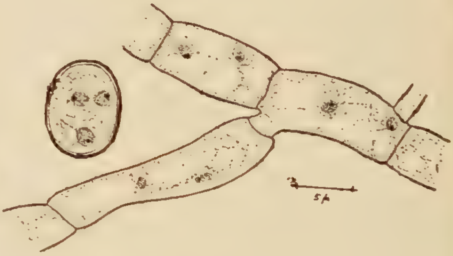

FIG. 1*b*

FIG. 1*a*. Unstained perithecial primordia formed on small vegetative hyphae in hanging-drop culture. FIG. 1*b*. A macroconidium and a portion of a mature vegetative mycelium showing the nuclear arrangement.

STRUCTURE AND DEVELOPMENT OF THE PERITHECIUM

Perithecia are formed after 4 to 5 days' growth at 27° C., normally in groups in localized areas. In petri-dish cultures they appear in the region of the inoculum after 4 to 5 days, occasionally around the edge of the plate after 10 days, and rarely appear elsewhere.

The perithecial primordium is similar to that described for other species, originating as a short, curved, lateral branch from a thin vegetative hypha. It becomes septate, and later is enveloped by outgrowths from its basal cells or from the vegetative hypha (Fig. 1*a*). Nuclear arrangement and possible cellular fusions of this stage have not been examined.

The enveloping branches form a wall which becomes differentiated into two zones. The outer zone is one to three cells thick, the cell-walls becoming slightly thickened and pigmented at an early stage. The inner zone is four to six cells thick, with thin walls and denser contents.

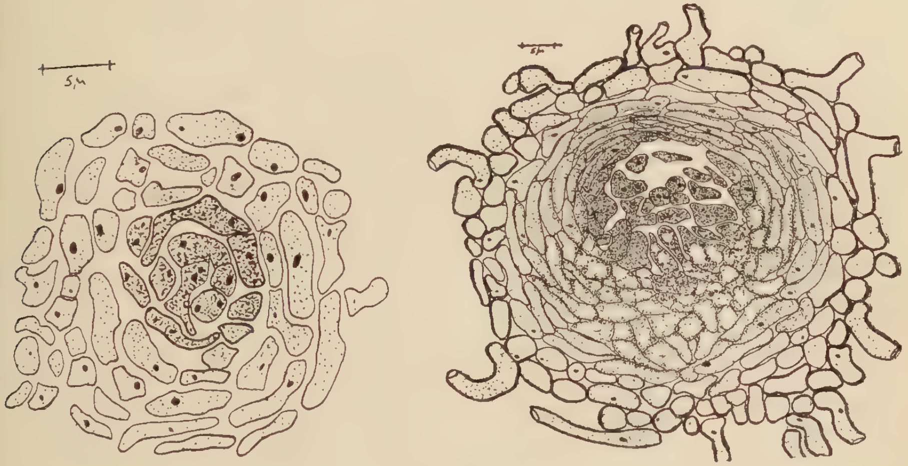

FIG. 2*a*

FIG. 2*b*

FIG. 2*a*. Section of a young perithecium showing linear growth of the ascogonium to form a darkly staining central coil. FIG. 2*b*. Section of a young perithecium showing the start of cushion cell and of neck formation. The fertile cells show traces of a linear arrangement distorted by growth in the confined space in the centre of the perithecium.

The ascogonium divides to form a coil of multinucleate cells, with dense contents staining more readily than those of the wall cells (Fig. 2*a*). The nuclei are occasionally in pairs, but more usually are scattered irregularly. In some sections the coil appears to be in close contact with the wall, there being no indication of a central cavity. In other perithecia it is coiled freely within a central cavity, there being a distinct space between the wall and ascogenous cells which is not due to shrinkage during preparation. The formation of a similar initial coil has been reported for *C. moniliformis* (Andrus and Harter, 1937), *C. fimbriata* (Gwynne-Vaughan and Broadhead, 1936), and *C. multiannulata* (Andrus, 1936), but has not been found in other species. The coil later alters in form, due to a change from the original regular linear division to a more irregular type with a tendency towards centripetal multiplication. The connexion between the cells is easily broken. While some remain in irregular rows the arrangement usually becomes much distorted by continued growth and overcrowding in the confined space in the centre of the perithecium (Fig. 2*b*). In sections more than 5 μ thick these cells appear as an almost solid mass, those in the lower portion merging with the inner wall. The majority have dense contents, but a number begin to form large vacuoles. By this stage the walls of the cells in the outer zone of the perithecium have become thickened and brittle, and the cells in the inner zone have commenced to enlarge and become vacuolate. This enlargement begins in the cells at the base of the perithecium and extends upwards on each side. By the time the outer zone has developed into the thick carbonaceous wall characteristic of the genus, these enlarged cells of the inner zone have developed into a broad band of 'cushion cells' (Fig. 3).

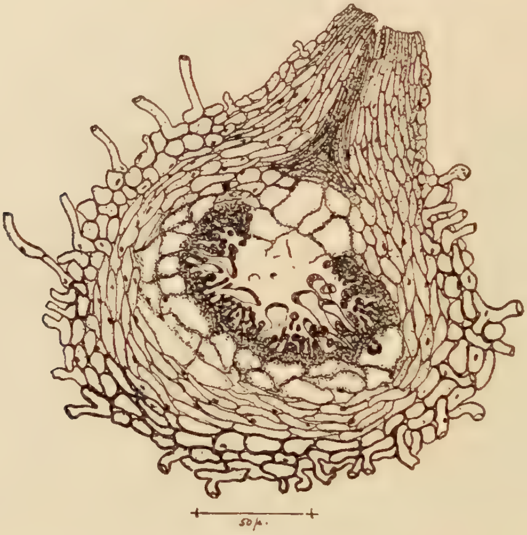

FIG. 3. Section of a perithecium showing the cup-shaped mass of fertile cells surrounded by a band of 'cushion cells'. The thin-walled tissue which originally filled the centre of the cup has begun to break down and to be displaced in the lower part of the cup by the club-shaped vacuolate ascogenous cells.

Further multiplication of the ascogenous cells takes place mainly at the sides of the mass, so that by the time the cushion cells have formed a complete investment they form a cup-shaped mass in the centre (Fig. 3). The tendency to radial arrangement is retained, though many irregularly scattered cells can also be seen. There is much variation in size, shape, and nuclear arrangement, but a gradual increase in the number of small binucleate cells becomes evident (Fig. 4*a*).

This division to form an irregular mass of multinucleate cells is similar to the corresponding stages in *C. Piceae* as described by Varitchak (1931), *C. fimbriata* as described by Mittman (1933), Andrus and Harter (1933), Gwynne-Vaughan and Broadhead (1936), and to *C. moniliformis* (Andrus and Harter, 1937) and *C. multiannulata* (Andrus, 1936) after the loss of the initial coil arrangement. These authors, however, disagree in the interpretation of their observations. Gwynne-Vaughan and Broadhead, and Varitchak, describe the divisions as resulting in 'ascogenous hyphae', though Varitchak states that these change form later in development and become difficult to identify. Elliott (1925) also interprets the cells of *C. fimbriata* as 'ascogenous hyphae', but it is noticeable that the drawings illustrating his article bear little resemblance to the microphotographs of similar stages of the same species figured by Gwynne-Vaughan and Broadhead. According to Andrus and Harter and to Mittman the loss of the cell-wall early in development strongly influences the further divisions of the ascogenous cells. They state that no definite hyphae are formed, though in *C. fimbriata* they report that the daughter-cells may cling together in 'rough chains'. This breakdown of the wall of the ascogenous cells is not found in *O. majus*. Though there is no distinct hyphal system after the initial coil has been lost, a definite wall can be demonstrated throughout the divisions leading to ascus formation. The walls of all cells within the outer layers of the perithecium are delicate but can be seen in a heavily stained preparation. The walls of the ascogenous cells exactly resemble those of the other thin-walled tissue in the perithecium, and of the vegetative hyphae, and are not lost until after maturation of the ascus. The irregular outline of the naked protoplast of the mature ascus is strikingly different from the outline of the young ascus, and from the outline of all cells before ascus formation (Fig. 6*g*). It is interesting to note that although Gwynne-Vaughan and Broadhead's description differs from that of Andrus and Harter, the microphotographs they publish are very similar to those of corresponding stages in *O. majus*. In both sets of illustrations distinct chains of cells appear, but no filaments resembling hyphae can be seen. In *O. majus* there is no indication of any localized growing-points, such as one would expect if hyphae were being formed, and the extremely loose connexion between the relatively unelongated daughter-cells is most unlike any normal hyphal system.

The irregular arrangement of the majority of nuclei is similar to the descriptions by more recent authors, there being no regular system of paired nuclei such as is drawn by Elliott (1925). The occasional 'pairs' are probably late stages in division, before the daughter-nuclei have moved apart.

Formation of the perithecial cavity. The formation of the cavity has received little attention from previous workers. It is detailed here for comparison with the method in another species which will be described in a later communication.

In the early stages the ascogenous cells occupy the whole of the centre of the perithecium, appearing as a solid mass in sections more than 5 μ in thickness. There is, however, a small amount of space above the cells, and between those in the lower portion (Fig. 2*b*). Later the centre becomes filled with a thin-walled tissue apparently continuous with the inner wall (Fig. 3). The vacuolate ascogenous cells already described enlarge greatly during development, their swollen heads displacing the thin-walled tissue at the sides and base of the hollow in the cup-shaped mass (Fig. 4*a*). Shortly before ascus formation the thin-walled tissue and the vacuolate cells begin to disintegrate (Fig. 4*b*). Breakdown is also apparent among the other ascogenous cells in the period immediately before ascus formation, some of the smaller ones not destined to form asci becoming vacuolate and losing their walls. By the time nuclear fusion has taken place in the ascus there is a large cavity in the centre of the perithecium, containing the disorganized remnants of these cells. During the maturation of the asci the cushion cells of the wall break down, the mature perithecium containing a mass of ascospores embedded in a mucilaginous mass.

This agrees with the findings by Andrus and Harter (1937) and Andrus (1936), who describe digestion of the inner wall and of certain ascogenous cells in *C. fimbriata*, *C. moniliformis*, *C. multiannulata*. Gwynne-Vaughan and Broadhead (1936) find no evidence of digestion in *C. fimbriata*, and attribute the enlargement of the cavity to loss of turgidity by the cushion cells. In *O. majus* the disorganization is due to digestion, there being a gradual breakdown of the cells rather than a sudden collapse due to loss of turgidity.

The large vacuolate multinucleate club-shaped cells which are formed during the period preceding ascus formation have not been described for other species of these genera. Andrus and Harter, however, describe fragile inflated and 'apparently uninucleate' cells preceding the asci in the centre of the perithecium, which are formed by certain types of division of the multinucleate fertile cells of *C. moniliformis*. In *O. majus* the club-shaped cells occupy the lower portion of the perithecial cavity shortly before ascus formation, the asci being formed between their lower ends. During the maturation of the asci these club-shaped cells disintegrate, their remnants joining the other disorganized tissue in the centre of the perithecial cavity. They are apparently analogous to paraphyses, though here formed by the ascogenous tissue. Brooks (1910) has described somewhat similar cells 'of irregular growth . . . poor in cytoplasm' developed from the ascogenous cells of *Gnomonia erythrostoma* in the period before ascus formation. He notes that they cannot be regarded as typical paraphyses, although they may take part in the formation of the cavity of the perithecium.

Formation of the neck. The first sign of neck formation is a slight increase in the density of the contents of some of the inner wall cells in the upper portion of the perithecium (Fig. 2*b*). This becomes visible at about the same time as the first enlargement of the cushion cells. These inner wall cells elongate and divide rapidly, forming a cone of filaments projecting towards the outside of the perithecium. By continued growth they force themselves between the cells of the wall immediately above them and protrude to the exterior by about the time the fertile cells have formed a cup-shaped mass in the centre (Fig. 3). This is purely a mechanical action, as there is no indication of any breakdown of the displaced cells. By continued growth the typical long cylindrical neck is formed, and the outer cells become thickened and pigmented. The inner cells break down to form a cavity which is continuous with the cavity of the body of the perithecium. The cells lining the base of the neck cavity separate from each other to form a fringe of filaments around the inner orifice. The outer neck hyphae remain in close contact, overarching the top, until after maturation of the ascospores. They then separate at the tip, and bend outwards to form the ostiolar corona of filaments. This entirely agrees with all previous accounts of neck formation in the genus.

FORMATION AND DEVELOPMENT OF THE ASCI

Asci are formed from the small binucleate cells, and cannot be distinguished from other ascogenous cells before nuclear fusion (Fig. 4*a*). There are no croziers, and in cases where the linear arrangement is not lost each of the cells in a chain may form an ascus (Fig. 5). This is similar to the method described for *C. fimbriata* by Elliott (1925) and Mittman (1933), and by Sartorius (1927) for *C. adiposum*, in that they agree that the ascus is formed without crozier formation. They differ in their accounts of the origin of these binucleate cells, Elliott and Sartorius finding them to be formed by the terminal septation of ascogenous hyphae, Mittman by the free division of isolated cells. In *O. majus* the mode of formation is intermediate between these two descriptions, some being irregularly arranged, but the majority growing in radial rows

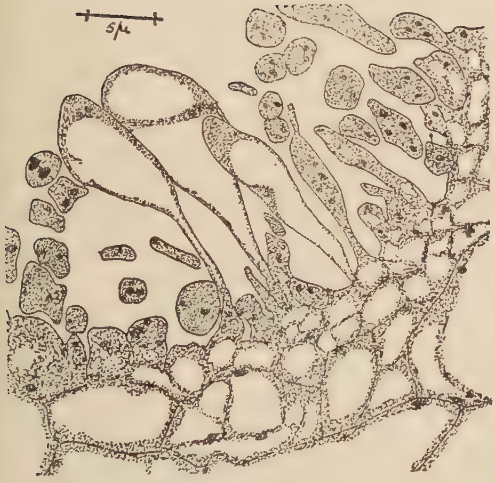

FIG. 4*a*

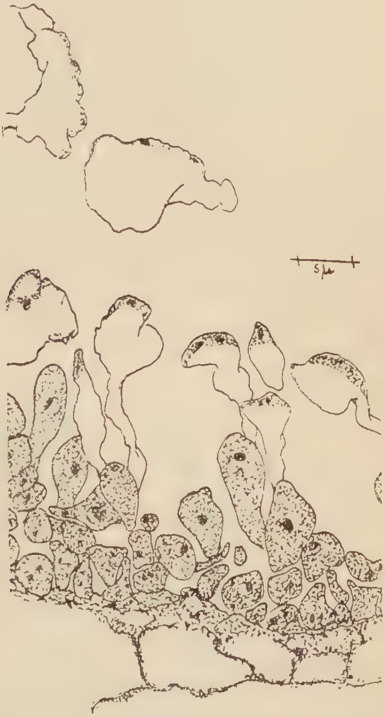

FIG. 4*b*

FIG. 4*a*. Part of the fertile cells in a section of the perithecium shortly before nuclear fusion in the ascus. Binucleate asci are present among the large multinucleate vacuolate cells. FIG. 4*b*. Part of the fertile cells in a section of the perithecium after nuclear fusion in the ascus. Uninucleate asci are present, and the multinucleate vacuolate cells are beginning to break down.

towards the centre of the perithecium. The description of hook formation given by Andrus and Harter (1937) is related to the loss of the walls of the ascogenous cells, and is supported by camera-lucida drawings of isolated figures from their preparations. A detailed study of the mass of ascogenous cells in *O. majus* has failed to show such stages. Groups bearing some resemblance to these figures could be picked out very occasionally, but in such cases their limits were very difficult to determine and there was a possibility of the section having been cut through some of the cells of the group. Such groups were of rare occurrence, while many of the binucleate cells could be seen to be formed in irregular rows by direct division. The figure given by Gwynne-Vaughan and Broadhead (1936), a simple curved hypha with four terminal nuclei, is unconvincing evidence of crozier formation, and differs very much from Andrus and Harter's description of the same species. The cells of the vegetative mycelium and the macroconidia are multinucleate, the nuclei having a hyaline vesicle with a darkly staining lenticular nucleolus on one side (Fig. 1*b*). It has not been possible to distinguish the details of the nuclear structure in the cells of the young perithecium, owing to their small size and to the dense contents of the ascogenous cells. In these stages the nuclei are seen as undifferentiated black masses (Figs. 2 to 4*a*), but after fusion in the young ascus the single nucleus enlarges, and again shows a hyaline vesicle with a lateral nucleolus (Figs. 4*b*, 5). The uninucleate asci remain in this state for a long time, and are very common in sections of semi-mature perithecia. At the end of this resting stage the appearance of the nucleus alters. A number of darkly staining masses are formed in the vesicle, which tend to run in irregular strands from the nucleolus to the opposite side of the vesicle (Fig. 6*a*). In some asci these strands seem to run into a second mass of chromatic material at this side. The appearance of this mass, if present, is uneven, there being no sharp outline such as delimits the nucleolus, and it probably represents merely the appearance of the closely packed ends of the strands.

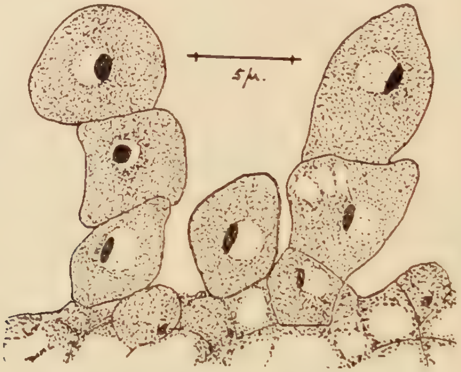

FIG. 5. Uninucleate asci showing linear arrangement.

Nuclear division takes place irregularly in the cytoplasm, and the daughter-nuclei are not grouped regularly in the centre of the ascus. The nuclei formed by the first and second divisions have a nucleolus and vesicle similar to the parent nucleus and lie quite freely in the cytoplasm (Fig. 6*b*, *c*). The nuclei during and for some time after the third division appear as minute black spots, only just visible under the $\frac{1}{12}$ in. oil immersion objective (Fig. 6*f*). The ascospores are formed by cleavage around the irregularly arranged nuclei after the third division. The nuclei increase to about two or three times their original diameter during the process and their vesicle and nucleolus can again be distinguished (Fig. 6*g*, *h*). The walls of the ascus break down during cleavage, the spores being held together in groups of eight by the residual protoplasm. They may break apart to be ejected singly from the ostiole, or remain in groups of two to eight. Spore formation takes place quickly once nuclear division has begun and there is little zonation of developmental stages in the perithecial cavity. This conventional course follows that described by

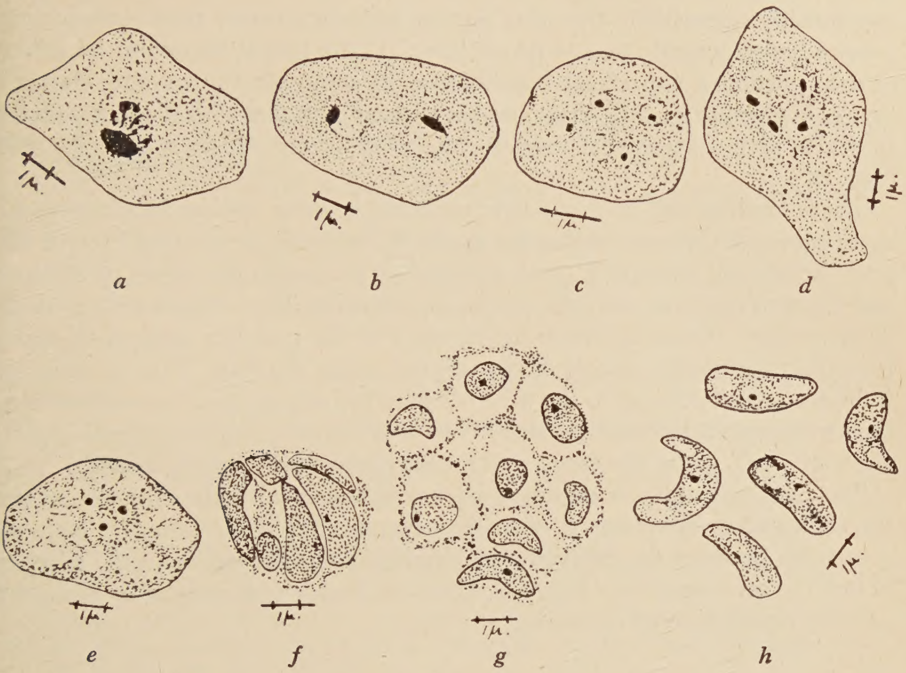

FIG. 6. Stages in nuclear division and spore formation in the ascus. *a*. Ascus in resting stage before the first nuclear division. *b*, *c*. Asci after the first and second nuclear divisions, showing the irregularly arranged daughter-nuclei, each with nucleolus and vesicle. *d*. Tetranucleate ascus in which the central grouping of the nuclei gives a slight resemblance to division within a central vacuole. *e*. Ascus showing nuclei in a central dense protoplasmic mass surrounded by a vacuolated layer. *f*. Ascus during spore cleavage, showing breakdown of the ascus wall, and very small spore nuclei. *g*. Section of an ascus at a later stage of spore cleavage, showing slightly enlarged nuclei and complete loss of the ascus wall. *h*. Young ascospores lying freely in the perithecial cavity.

Sartorius (1927) and by Gwynne-Vaughan and Broadhead (1936), there being no sign of division within the nucleoplasm (Elliott, 1923), or nuclear vesicle (Mittman, 1933; Andrus and Harter, 1937). The presence of a vesicle in all daughter-nuclei in the ascus supports the view that it is associated with each individual nucleus only, and that it takes no part in spore cleavage. The characteristic vacuoles which are formed during maturation of the ascus may occasionally aggregate in the outer portion to form a foamy layer surrounding a more dense central mass of protoplasm. If the nuclei chance to be in the central portion a superficial resemblance to some of Andrus and Harter's figures is given (Fig. 6*e*). The close association of nuclei with distinct vesicles may also give a superficial resemblance to divisions within a large vacuole (Fig. 6*d*).

These findings will be discussed in relation to other species in a later communication, but it is noted that the similarity between the general form of the perithecium and those of *C. fimbriata* and other species described by Andrus and Harter (1937) would lead one to expect similarity in the details of their development. There is, however, no sign of the peculiar method of spore formation within the vesicle of the original ascus nucleus. The presence of walls around all cells up to the time of spore formation shows resemblance to the account of *C. fimbriata* given by Gwynne-Vaughan and Broadhead (1936), although in *O. majus* the ascus wall breaks down during spore cleavage.

The tendency to linear septation seen at all stages up to the initiation of the asci indicates a relationship to normal ascogenous hypha formation. The loose connexion between the relatively unelongated daughter-cells and the absence of definite growing-points suggests, however, that 'ascogenous cells' is a more suitable description of the mass.

SUMMARY

1. A description is given of the details of perithecial formation in a new isolation of *Ophiostoma majus*, and the findings are compared with previous accounts of species of *Ophiostoma* and *Ceratostomella*.

2. It is shown that the ascogonium divides to form a compact mass of multinucleate ascogenous cells. These do not form definite growing-points, but a tendency towards linear septation can be seen.

3. The presence of a distinct wall is demonstrated in all cells within the perithecium up to the time of maturation of the asci. The mature ascus is shown to be deliquescent, the wall disintegrating during cleavage.

4. The nuclear vesicle is shown to play no part in spore wall formation.

5. The formation of the perithecial cavity is shown to be by the digestion of certain cells within the perithecium during the formation of the asci, combined with the breakdown of the cushion cells of the wall at a later stage.

6. A variation in perithecial size is described which is greater than that seen by van Beyma in the original description of the species.

ACKNOWLEDGEMENT

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